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FAO Fisheries Technical Paper No. 137
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FIRI/T137
MFS - Methods Water Pollution

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MANUAL OF METHODS IN AQUATIC ENVIRONMENT RESEARCH

Part 1 - Methods for Detection, Measurement and Monitoring of Water Pollution

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
ROME, 1975

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IN	Ediciones provisionales de los inventarios y listas de datos de pesca acumulados y guardados por el Centro de Datos Pesqueros de la FAO
CB	Listas de periódicos, secciones especiales de la «Aquatic Sciences and Fisheries Abstracts (ASFA)», bibliografías especiales y trabajos relativos a los problemas de documentación
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Algunos documentos tienen también otra identificación si, por ejemplo, son contribuciones a una reunión cuyos documentos han sido marcados con arreglo a otros sistemas.

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Rome, February 1975

Preparation of this Document

This Manual of Methods is mainly based on papers presented at the second FAO/SIDA training course, held in Göteborg and Stockholm from 31 July to 9 September 1973. It is divided into a theoretical part with papers on general aspects and problems of analysing aquatic samples and a practical part describing methods for measuring physical and chemical parameters, including the different classes of pollutants in the aquatic environment.

Distribution

FAO Department of Fisheries
Regional Fisheries Officers
Selector SM
Selector SI
FAO/SIDA Training Courses on
Aquatic Pollution

Bibliographic reference:

FAO (1975)
FAO Fish.Tech.Pap., (137):238 p.
Manual of methods in aquatic environment research. Part 1. Methods for detection, measurement and monitoring of water pollution

FAO manuals, aquatic environment research - methodology. Pollution. Chemical analysis - sediments, water. Oxygen demand, organic carbon, nitrogen, suspended matter, heavy metals, hydrocarbons, pesticides, methyl mercury. Analytic techniques - colorimetry, chromatography, spectroscopy.

PREFACE

Following the FAO Technical Conference on Marine Pollution and its Effects on Living Resources and Fishing (Rome, December 1970), collaboration between the Swedish International Development Authority (SIDA) and FAO was extended to aspects of training in the field of marine pollution and plans were developed for a series of Training Courses on Marine Pollution in Relation to Protection of Living Resources.

So far, two courses in this series have been held, and a third and a fourth course will be held in spring and autumn 1975.

The second course, held in Göteborg and Stockholm from 31 July to 9 September 1973, was arranged for research workers from developing countries (biologists, chemists, technicians, etc.) and was oriented toward planning and conduct of research and monitoring activities giving training in techniques and equipment currently used for the detection, measurement and monitoring of pollutants in the aquatic environment.

This Manual of Methods is mainly based on papers presented at the Training Course. It is divided into a theoretical part with papers on general aspects and problems of analysing aquatic samples and a practical part describing methods for measuring physical and chemical parameters, including the different classes of pollutants in the aquatic environment.

The contributions for the practical part have been completely rearranged and supplemented by Dr. S. Carlberg, Hydrographic Department, Royal Fishery Board of Sweden, to form a practical guide to measurement methods.

The technical editing of the Manual was done by Dr. S. Carlberg, with the assistance of Dr. R. Vaz, Wallenberg Laboratory, Stockholm. Final editing for style and consistency was carried out by the staff of the FAO Fishery Resources and Environment Division, particularly Dr. H. Naeve and Ms. M. Taylor.

The views expressed are those of the individual authors and contributors and not necessarily those of FAO or SIDA.

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1. STANDARDIZATION AND INTERCALIBRATION OF CHEMICAL METHODS IN OCEANOGRAPHY

by

Stig H. Fonselius

1.1 Introduction

Standardization and intercalibration of methods used in marine chemistry are of utmost importance. Marine chemists often wish to compare their results with results from other areas or with earlier results from the same area. Sometimes we are interested to find out how the conditions in a sea area were, e.g., fifty years ago. Of course, we like to know if the methods used at that time are comparable to the methods used now. Marine programmes are often carried out as cooperations between several countries or laboratories. It is important that comparable methods are used in the different laboratories. It is naturally best if the same methods can be used in all laboratories, but often it is difficult for laboratories to change their routine methods.

The fact that analytical methods are not comparable seems somewhat strange because a certain amount of an element in a fixed volume should give an absolute value. The very low concentrations of most of the elements measured make large demands on the accuracy of the analytical method. It has also to be remembered that the high "salinity" of the samples interferes with the analysis, masking the result. In addition, the performance of a chemical analysis on board a ship in a crowded laboratory under bad weather conditions may naturally differ from that in a well equipped laboratory on land. It is difficult to avoid contamination of samples through dust in the laboratory, grease on the wire and hands, etc. Many elements have to be analysed immediately on board. Samples which are preserved for later analysis may be influenced by chemicals added, adsorption on bottle walls, etc.

Intercalibrations of standard methods used in oceanography, carried out in different areas, have shown that, generally, differences occur which are larger than the accuracy claimed in textbooks. This is true, e.g., for chlorinity and oxygen titration using the Winkler method. Other parameters have been shown to give much larger differences. Especially the nitrogen compounds seem to cause difficulties.

1.2 Worldwide intercalibration

In Japan, much work has been done for preparing reliable standard solutions for intercalibration of phosphate, silicate, nitrite and nitrate. The work is led by Professor Sugawara at the Sagami Chemical Research Center. Stable standard samples in glass ampoules have been prepared and in 1970 a worldwide intercalibration of nutrient salts was arranged by ICES/SCOR. Samples were distributed to 55 laboratories in different parts of the world. Results were obtained from 44 laboratories. Of these, 41 sets could be used in a statistical treatment. Most laboratories used generally accepted standard methods for their analyses. The final report has at present not been printed and here only some preliminary results can be given.

1.2.1 Phosphate

In very few cases was the error greater than 20 percent. No significant difference could be traced between various methods. For concentrations around 0.2 µg-at/l the accuracy was found to be ± 15 percent, around 0.9 µg-at/l ± 6 percent and at 2.8 µg-at/l ± 2 percent[1].

1/ For explanation of unusual concentration units the reader is referred to chapter 4: Units used in Chemical Oceanography and Marine Chemistry

1.2.2 Silicate

This has a higher concentration in sea water, and gave at 4,5 µg-at/1 ± 4 percent as a relative error, at 45 µg-at/1 the error was ± 2.5 percent and at 150 µg-at/1 ± 6 percent. Only in a few cases errors exceeding 10 percent were found.

1.2.3 Nitrite

In all laboratories the classical Griess reaction was used. At 0.2 µg-at/1 the relative error of accuracy was ± 5 percent, at 0.9 µg-at/1, 2.5 percent and at 1.8 µg-at/1, 2.5 percent.

1.2.4 Nitrate

This was the most difficult parameter to analyse and several laboratories reported difficulties. In general, the relative error of accuracy was rather good, 0.5 µg-at/1 gave an error of 2 percent, 9 µg-at/1 3 percent and 30 µg-at/1 3 percent. The percentages given are valid for all laboratories at the low level, but only for about half of the material at the medium and high level, where the error in many cases exceeded 10 percent.

We have, however, to remember that the intercalibration was carried out on artificial samples in shore laboratories. The ampoules had been tested and shown to give unaltered results. In this kind of intercalibration, the error caused by the sampling procedure and handling of the samples on board the ships is excluded and it is by no means surprising that the results are rather satisfying. The results only depend on the analytical skill of the chemists and on the standard solutions used in the laboratories.

Most analyses have to be carried out immediately on board the research ships. Therefore, intercalibration of routine methods should be carried out on board the ships. This is best done by arranging intercalibrations out at sea, at some suitable station or in a port, where all participating ships meet. This kind of intercalibration meeting is difficult to arrange and enormously expensive; ship time costs money. In intercalibration tests, the participants of course try to work as carefully as possible but, in fact, we are not interested in results analysed with special care. We would like to know the accuracy of ordinary analyses carried out during routine work at sea. Nothing can of course be done to avoid that the analysts try to work more carefully than usually but it is, in any case, important to have the technicians who ordinarily carry out the analyses at sea to participate in the intercalibration tests. The ultimate purpose of the intercalibration is to find out if ordinary routine data from the laboratories give comparable results and how large the differences are.

1.3 Baltic intercalibrations

Some results from intercalibrations carried out in the Baltic between laboratories from different countries are given below. The results have been published as Unesco technical papers in marine science No. 3 and No. 9.

1.3.1 Salinity

The salinity (or rather chlorinity) determination was standardized already at the beginning of the century. Special burettes and pipettes were designed for the work, standard sea water was prepared and distributed internationally and special tables (the Knudsen tables) were computed for the work. This method (the Mohr-Knudsen titration) has been used over the whole world until some 10 years ago when the salinometer was commonly accepted as a routine salinity instrument.

In textbooks in oceanography an accuracy for the Mohr-Knudsen titration of ± 0.02 per mille is claimed. The intercalibration trials in Copenhagen 1965 and in Leningrad and Copenhagen 1966 have, however, shown that such an accuracy between different laboratories does not exist in reality. The precision between titrations of a sample carried out by an

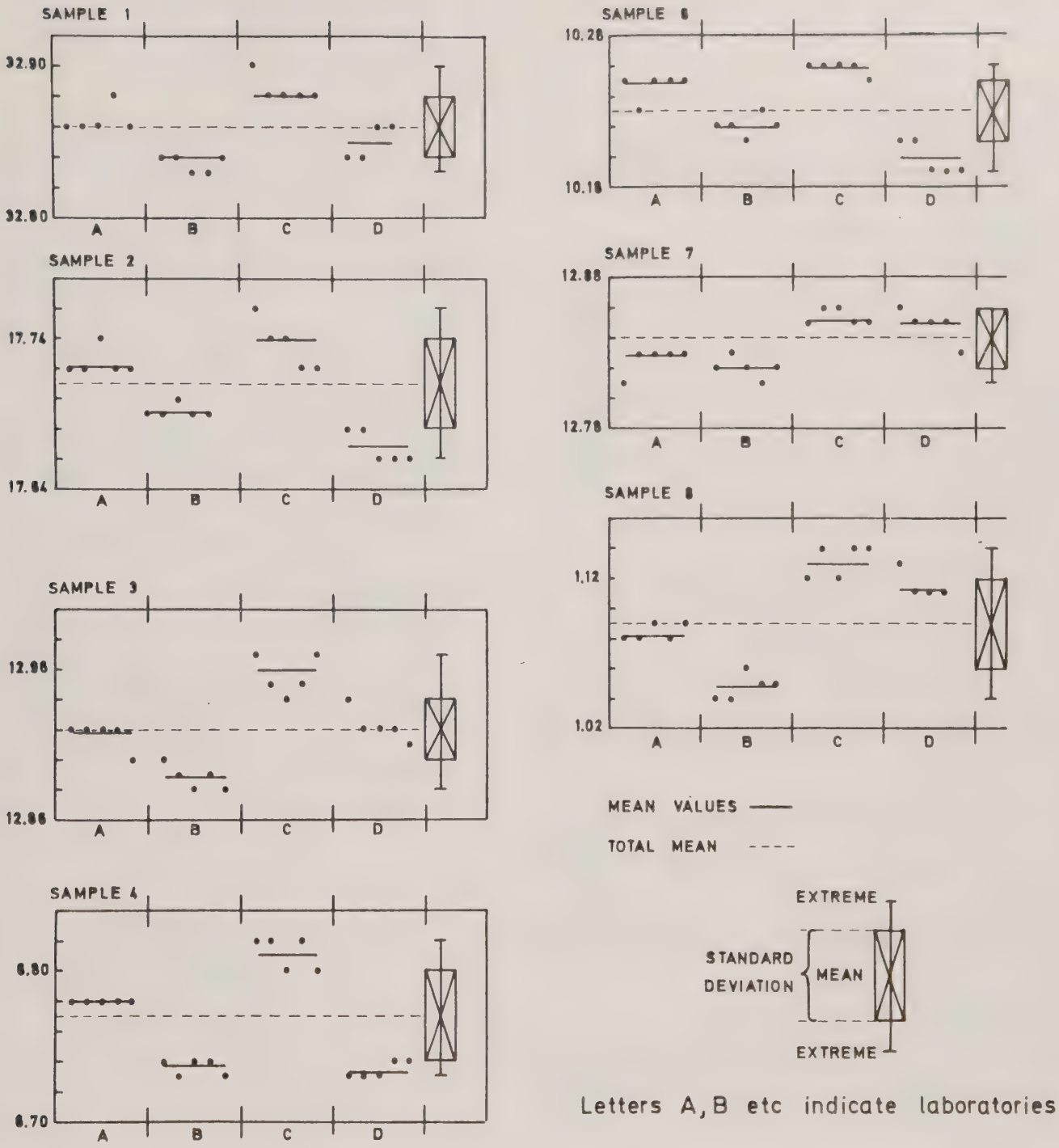

Figure 1. Salinity intercalibration results from the trial in Leningrad 1966. Salinity calculated from Chlorinity (Acc. Mohr-Knudsen tables)

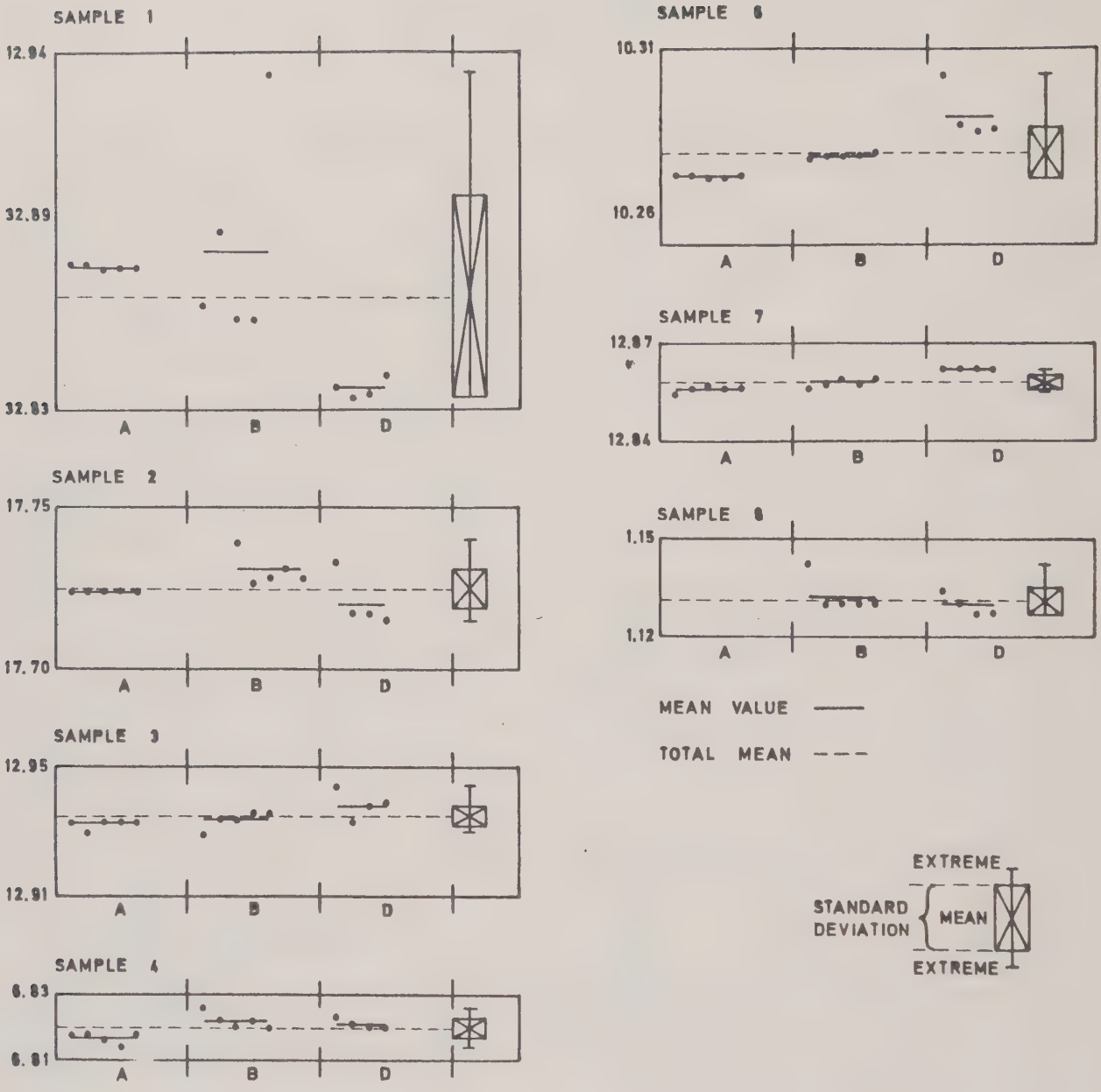

Figure 2. Salinity intercalibration results from the trial in Leningrad 1966. Salinity measured by Conductivity

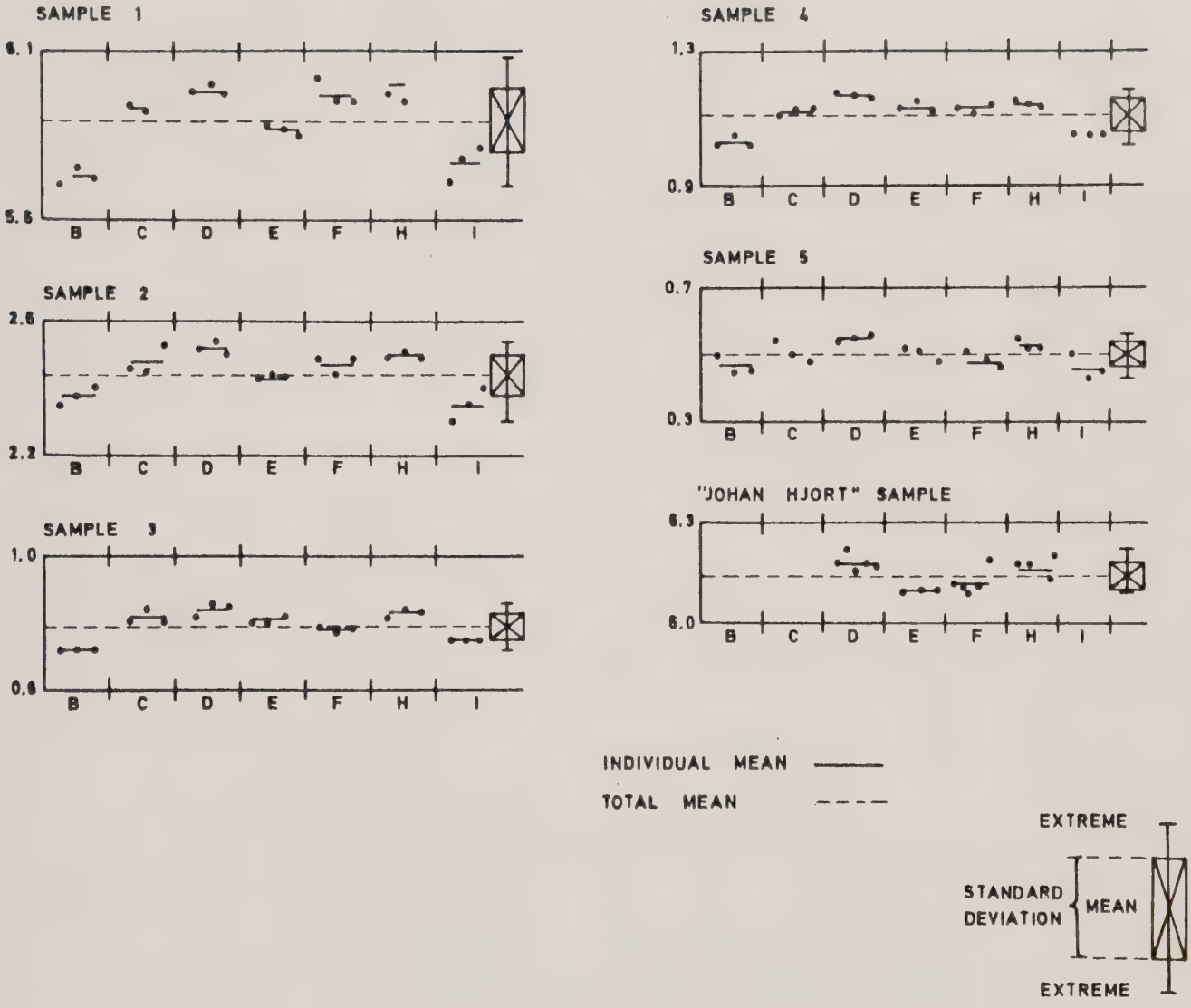

Figure 3. Results of the oxygen intercalibration in Copenhagen 1966

Table I

Modifications of the Winkler method used in the oxygen intercalibration in Copenhagen, 1966

Ship inst.	Manganese salt	Alkaline iodide	Acid	Sample volume	Vol. (ml) reagents	Vol. for titration	Reaction and settling time
D	$MnCl_2x4aq$ 400g/l	KOH + KI 300g+600g/l	H_2SO_4 50%	50 ml calibr.	0.5+0.5+1	All	Max. 10 h
H	$MnCl_2x4aq$ 830g/l	NaOH+NaI 335g+650g/l	H_2SO_4 50%	50 ml calibr.	0.3+0.3+1	All	Prec. settled to $\frac{1}{2}$ of bottle
B	$MnCl_2x4aq$ 400g/l	NaOH+KI 360g+150g/l	HCl conc.	125 ml	0.5+0.5+1	50 ml by pipette	24 h (invert. after 6 h)
I	$MnCl_2x4aq$ 400g/l	NaOH+KI 360g+150g/l	HCl conc.	125 ml	0.5+0.5+1	100 ml by pipette	min. 3 h
C	$MnCl_2x4aq$ 400g/l	NaOH+NaI 400g+900g/l	H_2SO_4 conc.	100 ml calibr.	0.5+0.5+1	All	24 h (min. 1 h)
E	$MnCl_2x4aq$ 400g/l + 4 ml/HCl	NaOH+KI 360g+100g/l	H_2SO_4 50%	125 ml calibr.	1 + 1 + 2	All	2 - 3 h
F	$MnSO_4x1aq$ 380g/l	NaOH+KI 500g+300g/l	H_2SO_4 conc.	125 ml calibr.	1 + 1 + 1	All	Prec. settled to 2/3 of bottle

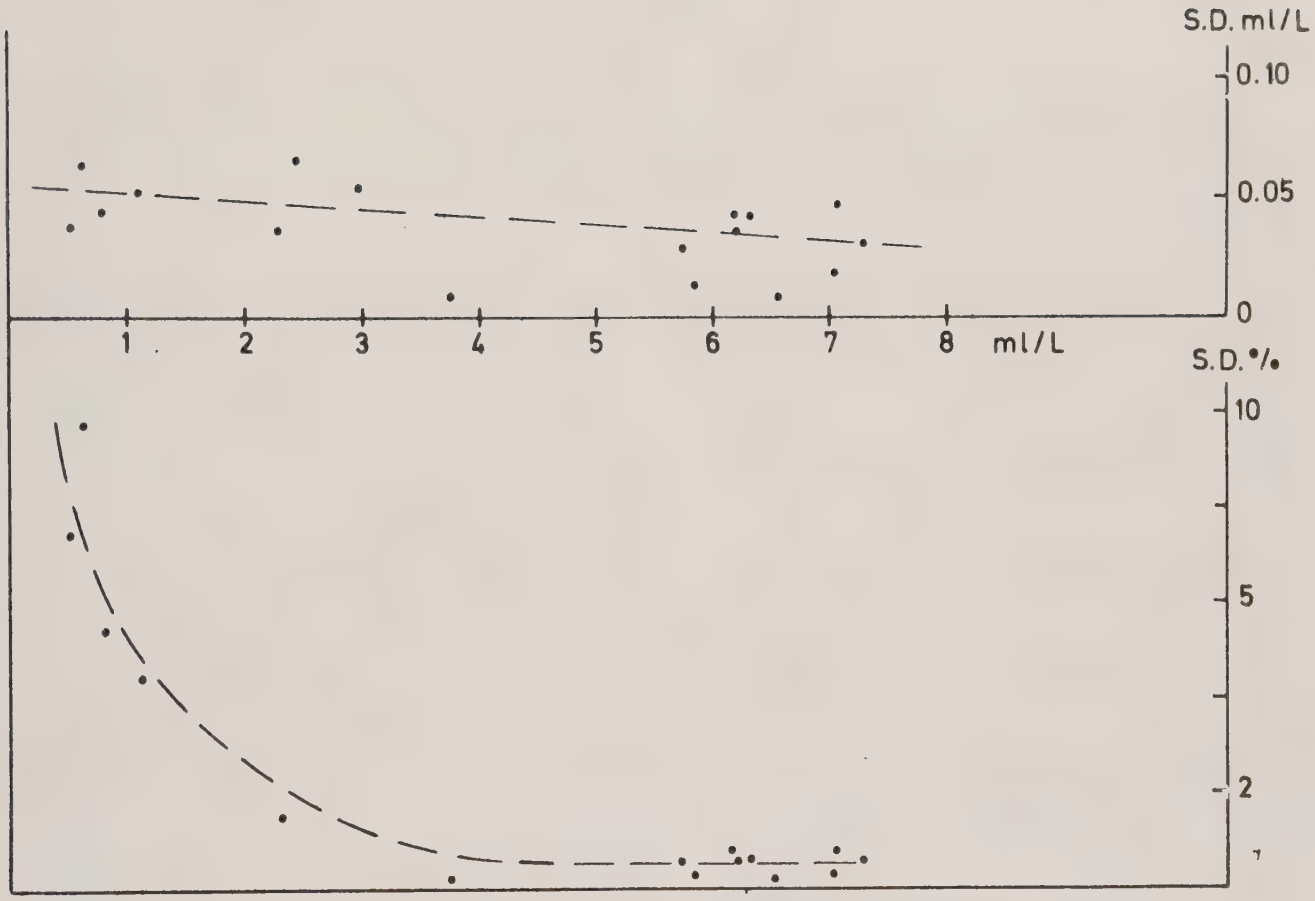

Figure 4. Standard deviations from the three oxygen intercalibrations in the Baltic and North Sea areas

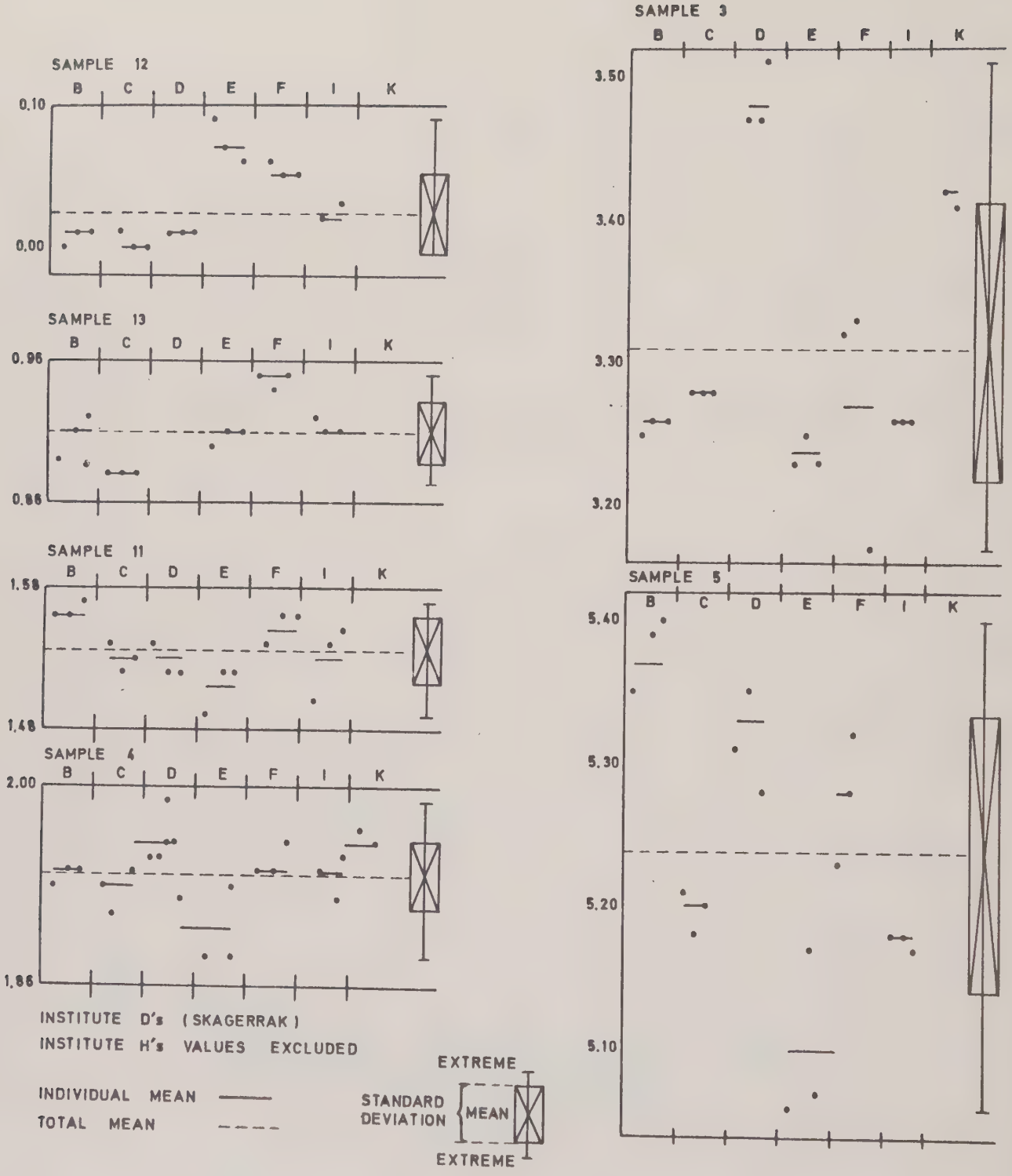

Figure 5. Results of the phosphate intercalibration in Copenhagen 1966

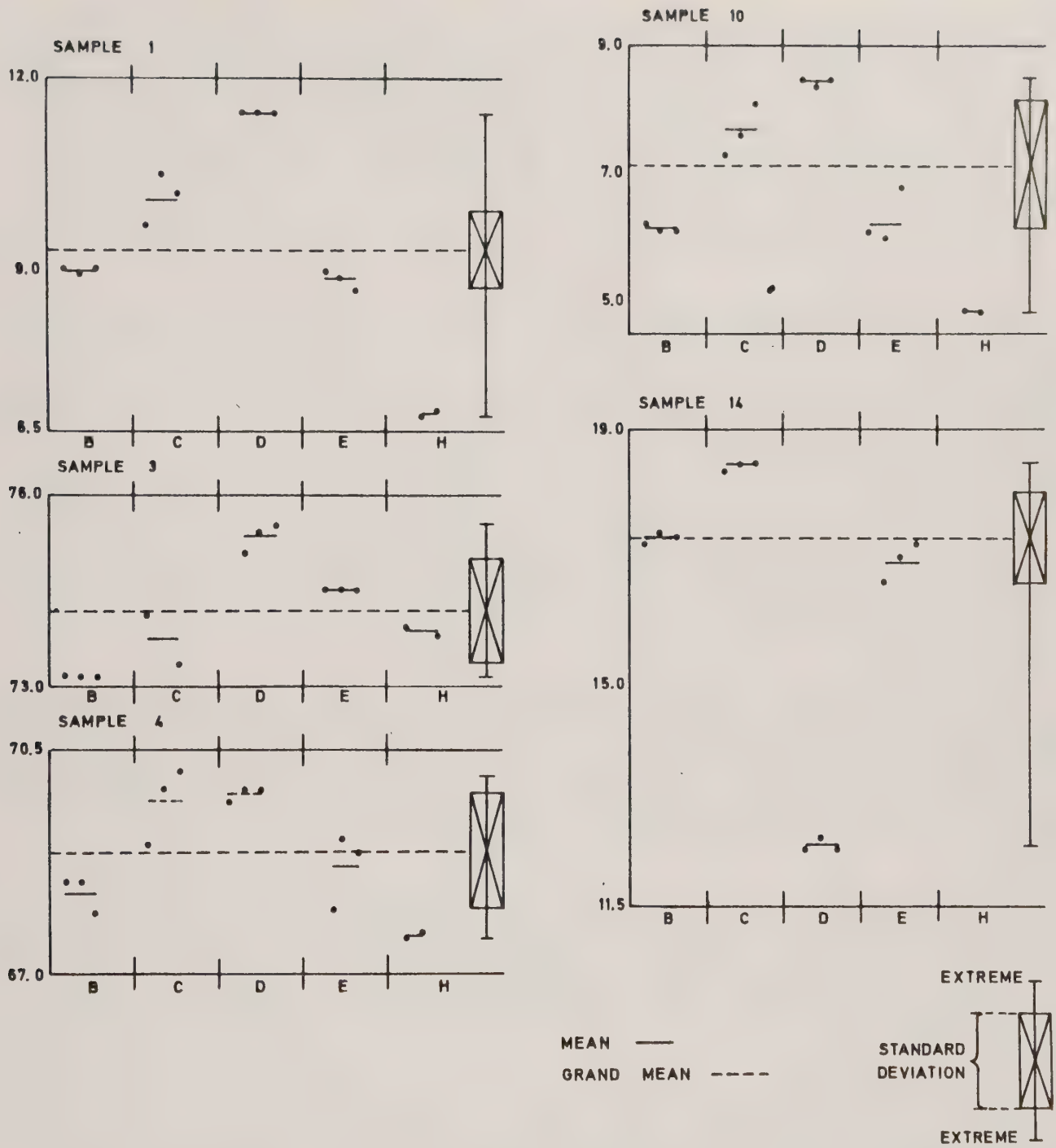

Figure 6. Results of the silicate intercalibration in Copenhagen 1966

analyst, may be excellent but there are considerable differences between laboratories. This seems to be mainly due to the calibration of the burettes and the cleanliness of the glassware. Figure 1 shows salinity intercalibration results from the trial in Leningrad 1966. We can, in some cases, see an excellent agreement between the individual analyses of a sample titrated by the same person but, in spite of the Copenhagen standard water, the results between the laboratories differ considerably. Figure 2 shows the results of the salinity determination by help of salinometers. Only three participants used salinometers, all of the same principle construction. The agreement is better than for titration, except for sample 1, where something seems to have gone wrong.

1.3.2 Oxygen

In oxygen intercalibration there is always the risk of contamination of the samples by atmospheric oxygen. Special precautions have to be taken. Figure 3 shows the result of the oxygen intercalibration in Copenhagen 1966 with seven participants. All used the Winkler method with different modifications (Table I). Figure 4 shows the standard deviations from all the three intercalibrations arranged in the Baltic and North Sea areas. The upper diagram in the figure gives the standard deviations in ml/l for the different samples. The standard deviations indicate that the results are almost independent of the oxygen concentration in the samples. Only a slight increase of the deviation can be observed with decreasing oxygen values. This is probably due to the titration error or "drop error", which of course will increase with the decreasing amount of thiosulphate used. The standard deviation expressed as percentage of the oxygen concentrations are shown in the lower diagram. This deviation will, of course, increase hyperbolically with decreasing oxygen values if the standard deviation in ml/l is constant. This may give the erroneous impression that the samples with low oxygen content have been contaminated by atmospheric oxygen during the sampling or analysis procedure.

From Figure 3 it can be seen that participants B and I got considerably lower values than the others. These two laboratories analysed 50 or 100 ml aliquots of their samples and they transferred the aliquots by pipetting. This method has been shown to give too low results due to evaporation of iodine during the pipetting procedure (Carritt and Carpenter, 1966). This error will grow with increasing iodine content of the sample. Grasshoff (1966) found that too low values will be obtained due to loss of iodine during the titration if the iodine content of the sample is low too. He recommends 600 g KI/l in the alkaline iodine reagent.

Carritt and Carpenter (1966) recommend a final pH of 2 in the samples. In coastal waters, where the salinity - and hence the buffer capacity of the water - varies considerably, the solubility of the oxygen may also vary much even at the same temperature. Therefore, it will be practically difficult to keep a pH of 2 in every sample. It will be necessary to have an excess of acid in the samples. Probably it is enough to use only 0.5 ml of concentrated sulphuric acid in a 100 ml sample instead of 1 ml, as recommended by Jacobsen et al. (1950).

1.3.3 Phosphate and silicate

Also nutrients were intercalibrated during the trials in the Baltic area. Some results of the phosphate and the silicate intercalibrations are shown. We are not successful with the nitrogen compounds and probably much research work has still to be done before we will get reliable methods for these compounds. Figure 5 shows the results of the phosphate intercalibration in Copenhagen 1966. We can see that the high phosphate concentrations caused difficulties, most probably due to the difficulties to read the high absorption values on the spectrophotometers.

Figure 6 shows the silicate results from the same occasion. It has to be observed that the concentration of the silicates is about 10 times higher than the phosphate concentration and that the scale therefore is 10 times smaller.

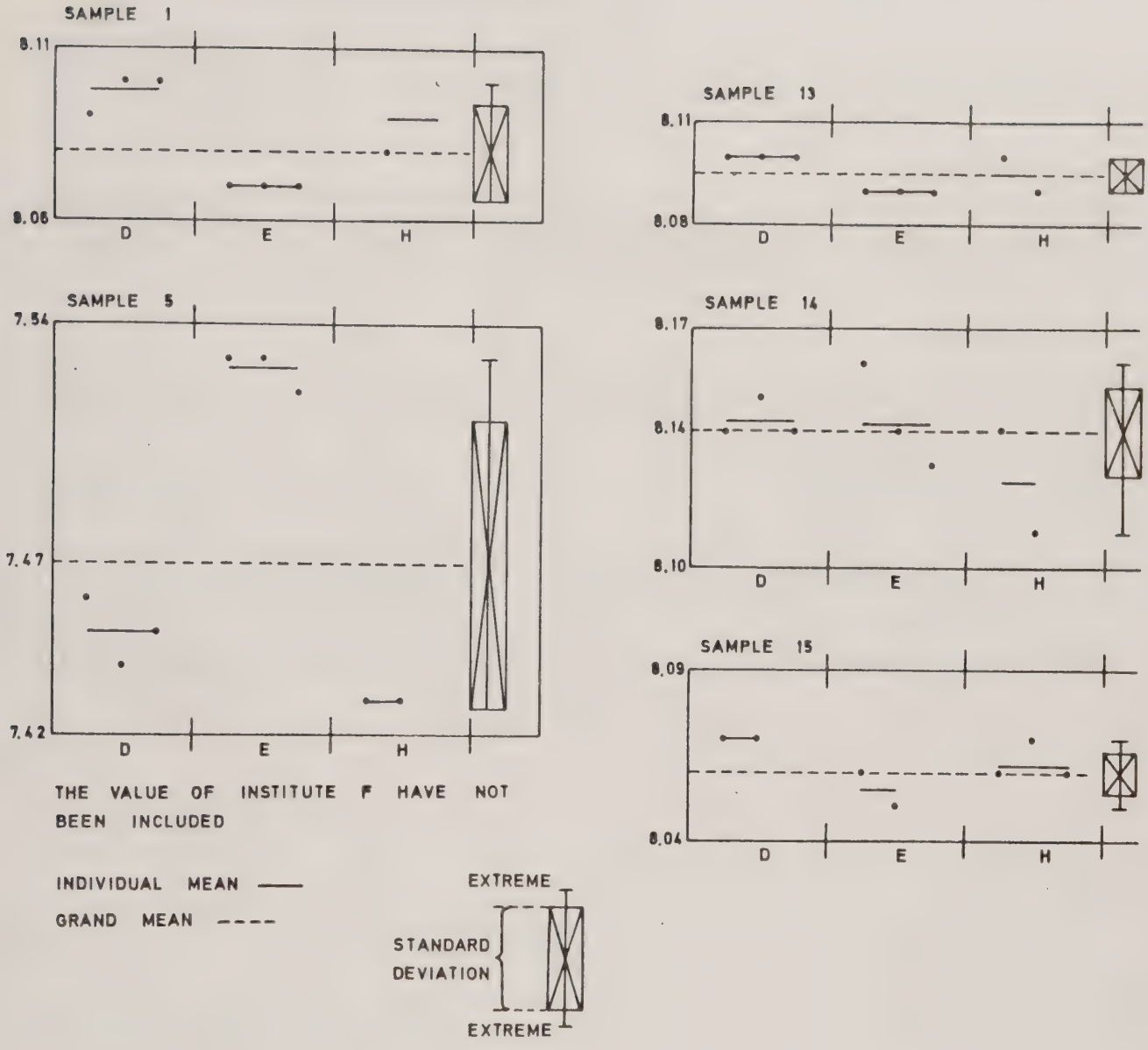

Figure 7. Some results of the pH intercalibration (in situ measurements)

1.3.4 pH

Finally, some results of pH intercalibration are shown (Figure 7). It was found that it is necessary to measure the pH at a constant temperature in a closed system in order to avoid contamination by atmospheric carbon dioxide.

1.4 Conclusions

The results from these intercalibration trials show that it is extremely important to arrange intercalibration of the methods before international cooperation programmes. If the results are not comparable, it will be impossible to draw conclusions from the results reported by different laboratories. When possible, standard methods should be used.

1.5 References

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2. STANDARDIZATION AND INTERCALIBRATION IN RESIDUE ANALYSIS

by

Sören Jensen

2.1 Internal standard

There is in residue analysis a great need for internal standards. It is difficult to measure accurately the small volumes remaining after evaporation; and the volume which can be injected in the gas chromatograph seldom exceeds 10 µl. When an internal standard is available it is not necessary to measure volumes if this standard is also present in the standard solutions. Such an internal standard must belong to the same class as the substances to be analysed and it should have a retention time not interfering with these. If the internal standard is added to the sample it can be used as a control for the whole procedure (recovery). In residue analysis, however, the levels of the chlorinated hydrocarbons may vary by magnitudes of ten from one sample to another. This means that very often the extract has to be diluted to an extent where the internal standard is not detectable. A better alternative is to estimate recovery percentages for different samples in the development step and only use the internal standard to control volumes. This is done in the following way: When the solvent is evaporated after extraction to estimate the fat, solvent containing the internal standard in a known concentration is added. It is often suitable to use 1 ml of solvent per 25 mg of fat. All further dilutions which might be necessary are done by means of the solvent containing the internal standard in the same concentration. Instead of measuring the injected volumes on the gas chromatograph, the peak height of the internal standard is compared to the corresponding peak height in the standard solution. A compound which has been proved for two years in residue analysis is 1.1-diphenyl-dichloroethene (DPDE). The concentration is 0.04 ng/µl.

Conclusion: The addition of DPDE to all final solvents used makes quantification more accurate.

2.2 Analytical accuracy

With residues around 1 ppm the accuracy is highest but with increasing levels, there will be a dilution factor to take into account. On the other hand, very low residues will give a bigger uncertainty because many steps are often necessary. Many reports on residue analysis state that the accuracy in the methods is above 85 percent. If no systematical error is involved the residues reported are below the true one.

Conclusion: The accuracy is most often below 100 percent and a result around 85 percent could be regarded as fairly good.

2.3 The necessary analytical sensitivity

This point is impossible to answer from a pure analytical point of view. If the question is to evaluate biological significance it has to be answered by biologists. If, on the other hand, the investigation is brought about in a monitoring system, experience from pilot studies have to tell which levels are necessary to make it possible to reach intelligent conclusions from the results.

2.4 Analytical methods in the future

Better electronics will possibly increase the sensitivity of the detector a little more. The question is, however, is it reasonable to study substances present at levels much below those possible today to detect by means of the electron capture detector (see also Widmark's microcosmos theory). Better gas chromatographic columns (capillary) will resolve many peaks today overlapping each other and thus increase the certainty of the result. Automation of the final steps in the analysis, the gas chromatographic one, is in good progress. There are

fully automated instruments on the market today. Such a one, working at the author's laboratory for more than one year, analyses 36 samples a day by means of automatic injections. If the samples are not too complex in composition, the instrument, via an electronic integrator and a teletype, can give full details of a chemical analysis calculated by means of a computer. If the sample composition is too complex, it is still necessary to "identify" and measure the peaks manually. These data are then printed on the teletype by hand. With better resolution on columns it might be possible to analyse all samples by means of fully automated instruments.

2.5 Conservation and transport of samples

If the aliphatic chlorinated hydrocarbons have to be taken into account in the analysis, all glassware has to be cleaned, not only by washing, but also with steam distillation. All glass containers are filled with distilled water and boiled (in a water bath) until about 10 percent of the added water has evaporated. The remaining water is drained and the glass container is closed immediately.

The best method is to always deep freeze the samples immediately after sampling and work them up (extraction) within a few days. It has been reported that, especially in liver samples, DDT can, to some extent, be converted to DDD. The same has occurred in samples which have been stored deep frozen for many months. If a preservation is necessary, formalin has been found to give no artifacts in the analysis. Water samples have to be sampled in glass containers and never in plastic; the surface of which may extract the chlorinated compounds from the water. To the water either mercury chloride or a few drops of chloroform per litre can be added.

2.6 Intercalibration and preparation of standard solutions

When a group of laboratories is occupied with residue analysis of organisms from different regions in a joint programme there is a great need for a system by means of which it is possible to ensure that the results are reliable, independent of where the analyses have been carried out.

The following three items have to be discussed: (a) standardized analytical methods; (b) conventional recovery experiments; and (c) intercalibration.

2.6.1 Standardized methods

In the past, many attempts have been made to standardize the methods used in residue analysis but most often these have been unsuccessful. Standardized methods will possibly only work well within smaller groups of equally trained persons where modifications can be brought about rather quickly. It will always be difficult to choose the best among all reported in the scientific literature at a certain time and often a slow-working, standardizing committee is formed.

When a certain method has been chosen, a rather long time will elapse before all laboratories involved in the programme have become well acquainted with the method. Due to the rapid progress of analytical chemistry, a standardized method will therefore often become old fashioned in the meantime or it will tend to retard the progress.

For the above-mentioned reasons many analytical chemists today strongly recommend against the use of standardized methods. On the other hand, it might be wise that a small group of well trained experts can meet and, from the existing methods, recommend a few to be used by laboratories which are starting the programme from scratch until they have gained their own experience.

2.6.2 Conventional recovery experiments

Almost every laboratory concerned with residue analysis will prove its method under development in some sort of recovery experiments and only start to analyse realistic samples when the recovery of added pesticides to biological samples is above, for instance, 80 percent. The loss due to the method is often included in the calibration curve or in the report where the values are stated to be uncorrected.

A conventional recovery experiment often includes the following steps: A homogenate of the organism to be analysed is divided into two portions (a) and (b). To (b) is added a known amount of the pesticides to be analysed and then (a) and (b) are analysed in parallel. To a test tube (c) is added the same amount of the standard as to (b). The standard is then diluted to the same volume as is the final one for the samples going through the method. A recovery experiment is said to give 100 percent recovery if the result from (b) minus the result from (a) is equal to the result from (c). Recovery experiments, as mentioned above, may give a systematical error for the following reasons: When the substances to be analysed are added to the homogenized biological sample *in vitro* they will be situated on the outside of the cells from where they might be easier to remove by means of a given solvent with a low elution power than if the pesticides were given to the animal *in vivo*. This risk is smaller when solvents, such as acetone, methanol or mixtures thereof with ether, are used. In these cases, in contrast to hexane-extraction of dried material, the cells are burst open and thus make the intracellular substances, fat and pesticides available for the solvents.

In the past, hexane has very often been used because it extracted less fat and, as a consequence, a minor clean-up process is needed. A whole conventional recovery experiment will never disclose whether naturally intaken substances are also extracted quantitatively.

To the best knowledge of the author, the only way to avoid objection to recovery experiments is if the substances to be tested for recovery in a new method are given to living organisms and in the form of radioactive marked ones. It is then possible to estimate the true values on an absolute basis with an independent method not suffering from the drawbacks mentioned for the conventional recovery experiments. Of course, this method will not reveal anything about the formation of metabolites during the passage through the animal.

2.6.3 Intercalibration

When no standardized method is available, the need for an intercalibration system increases. This system must include the identification and estimation of the substances to be analysed. Even on an international basis many bad intercalibration programmes have been set up in the past. It is not just enough to homogenize a biological sample from a certain organ and send it to all laboratories. This method suffers from the lack of knowledge as to what substances are really present and in what quantity. As an alternative to this system, spiked samples have been used where the substances to be analysed are added to a non-contaminated sample of, for instance, cod- or corn-oil. Here both the composition and the levels are known. On the other hand, a good result in this system will not necessarily be the same as a good result when the same method is used on a realistic sample for the same reasons as mentioned under "Conventional recovery experiments" above. Here, it is also preferable to work out a method where traced compounds are added to the test organism *in vivo*.

Important international intercalibrations have been carried out in this field organized by OECD (Holden, 1970) and ICES.

2.7 Fresh-weight and fat-weight basis

Most investigations up to now have reported the residues in organisms on fresh-weight basis and this is natural when the main interest lies in the food hygiene plane.

From an ecological point of view, however, it is much more valuable to refer fat soluble substances, such as the chlorinated hydrocarbons, to a fat-weight basis. It is then easier to refer to organisms on the same trophic level independent of the fact that one species might be fat and another not. Many fishes also have a variable fat content during the year. If fat is used as reference it is important that the fat estimation method, which the different laboratories use, gives the same fat percentage when the same sample is analysed. This estimation must also be included in the intercalibration.

2.8 Standard solutions

To be sure that all analyses in a monitoring system are reliable, it is necessary to produce standard solutions. This can be done at a few laboratories and the final solutions cross checked. Often the stock solution has a concentration of about 100 times above the concentration used in routine injection. These stock solutions must immediately be transferred to ampoules and sealed by means of an oxygen flame.

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3. ERRORS AND ACCURACY IN CHEMICAL ANALYSIS WITH SPECIAL REFERENCE TO MARINE CHEMISTRY

by

Stig H. Fonselius

3.1 Introduction

Generally, we judge an analytical chemist on his skill, that is, his ability to produce correct analyses. The maxim that no chain is stronger than its weakest link holds also for quantitative chemical analysis. The most exact analytical work, and the most sophisticated techniques and instrumentation, cannot give pricise and reliable results if something is wrong with the sample. It may have been altered by changes in composition before it reached the laboratory or it may have been contaminated during the sampling. Therefore, it must be stressed that the sampling procedure is the most important step in analytical work.

The chemical oceanographer should realize that the constituents in his sample have a three-dimensional distribution pattern. The distribution pattern in the marine environment is, in contrast to the distribution in a mineral resource, influenced by chemical, physical and biological processes which may result in a more or less pronounced variability in the distribution at the sampling moment. Normally, only the main features of this distribution are known before the sampling. Therefore, the general rules for the sampling of ores, minerals, gases, etc., cannot be applied directly. It is necessary to know and understand the environment and the variety of processes which may influence the sample. The marine chemist should, therefore, also have a good general knowledge of oceanography.

The analytical chemist tries to employ precise and reproducible procedures in his work. The marine chemist, however, is faced with a highly variable environment over which he has no control. The analytical chemist views errors mostly as mistakes which he can avoid. In sea-water sampling procedures, the error cannot be completely eliminated. It can, however, be diminished by applying improved sampling methods by taking large numbers of samples or by using large samples. The marine chemist should always ask himself how representative the samples are and how large the sampling error is. In addition, the analyst should remember that the exact sampling site can seldom be determined and that the sampling depth may be somewhat uncertain.

3.2 Systematic errors

One distinguishes between two types of errors, the systematic error (or bias) and the random error. The systematic error always leads to a result which is either constantly too high or too low, compared to the true value. The systematic error may be introduced in the sampling procedure through contamination of the sample by the sampler, absorption of matter into the sampler's walls, or errors in the analytical method itself. Another source of error in, for example, oxygen analyses, is the contamination of the sample by atmospheric oxygen during the drawing of the sample from the sampler, or losses of iodine vapour during transfer to the titration beaker.

Errors in the metric depth, caused by wire angles or faulty unprotected thermometers, are also a frequent source of bias. The systematic error is the most serious form of error because it will appear in all samples taken by the same method. It can only be detected by changing the sampling method. Often that is not possible for practical reasons. The sampling cannot generally be repeated at the same spot and there is no guarantee that the same water will be sampled. Depletion or contamination of the sampled water during the storage period in the sampler can be detected by performing time series experiments on the constituent to be analysed. The only way of testing equipment for systematic error in sampling is to attach different types of samplers to the hydro wire as close together as possible. Even then, the stratification in the water may cause errors. Different samplers may trap water from different water layers. We do not know the true value and it is therefore difficult to detect systematic errors. It is sometimes possible to detect bias in sampling with the help of T-S diagrams. In such cases the water in the water column must have a stable stratification.

Therefore, the samplers and sampling techniques should be carefully examined before using them for sampling. Under field conditions, some of the systematic errors can never be completely avoided. Such errors are, inaccurate navigation, improper depth of the samplers, and effects from the rolling of the ship. These errors must be accepted but it is important that the marine chemist is aware of them and, therefore, able to estimate the bias.

3.3 Random errors

Random errors, or determinate errors, are the second type of errors which may affect the result of a chemical analysis. Random errors give results which will sometimes be higher or sometimes lower than the true value. These errors are caused by personal errors, instrumentation and maybe bad flushing properties of the water sampler. In most cases, it is possible to detect random errors by comparing independent analyses from the same sample using spiking methods. Random errors are always present in analysis and have no relation to the bias which may be present or absent. Their magnitude can be reduced by repetition of the analysis.

3.4 Precision and accuracy

Analytical chemists use two terms for describing error; precision and accuracy. Precision indicates how closely the results of a set of independent analyses of the same sample agree. Accuracy is the deviation of the analysis from the true value. The accuracy is therefore a measure of the total error. An analytical result is not accurate unless both systematic and random errors are small. It is obvious that precision is easier to express numerically but that accuracy is the more informative measure of error.

3.5 Specificity and sensitivity

In analytical chemistry we still have two more factors which influence the results. The specificity of a method states how specific it is for the element analysed. There are often other elements which also react in the same manner with the reagents used and may thus influence the result. For example, in the chlorinity titration using the Mohr-Knudsen method, not only do chloride ions react with silver ions, but iodine and bromine are also precipitated as silver salts.

As a rule, few analytical methods are absolutely specific and generally the chemist must be content to obtain a reasonable degree of specificity for his analysis. The information on specificity, therefore, can hardly ever be complete but, the longer a particular method is used, the more information will be gained on its specificity.

The sensitivity of a method may be defined as the smallest positive amount of substance greater than zero that can be measured by the method used.

3.6 Definition of the terms precision, accuracy, specificity and sensitivity

Thus, we have four expressions with different meaning; precision, accuracy, specificity and sensitivity. Precision expresses how much several analyses of the same sample differ from each other. Accuracy expresses how much the obtained value differs from the true one. Specificity expresses how much other elements interfere in the analysis. Sensitivity expresses the smallest amount which can be determined by the method.

3.7 Definitions of the most commonly used statistical terms

We know from experience that when a sample is analysed by many analysts or when one analyst analyses the same sample many times, we get values that differ from the mean value or observed mean μ. The mean value $\mu = (x_1 + x_2 + \ldots x_n)/n$, where x_1, $x_2 \ldots x_n$ represent values on either side of μ, they are the observed spread of the analytical result. These values deviate from the mean (μ). The square of this deviation $(x - \mu)^2$ can be calculated for every individual result and the mean value of the squares $[(x_1 - \mu)^2 + (x_2 - \mu)^2 \ldots (x_n - \mu)^2]/n$ is called the variance (V), because it measures the variation of the observations.

The square root of V is expressed in the same units as the observations and is called "the standard deviation". It is usually denoted by δ and hence:

$$\delta = \pm \sqrt{V} \text{ or } \delta^2 = V$$

The expression "representative determinations" can be defined as the determination that gives a value that differs from the average value (μ), representing a large number of individual determinations (x), by one standard deviation δ_x

$$\delta_x = \pm\sqrt{\left[\sum_1^n (x-\mu)^2\right]/n}$$

or if the number of replicate determinations (n') is small, an unbiased estimate, (s_x), of the standard deviation is given by:

$$s_x = \pm\sqrt{\left[\sum_1^{n'} (x-\bar{x})^2\right]/(n'-1)}$$

where $\bar{x}$ is the arithmetic mean of n' replicate determinations. The standard deviations should be considered as a measure of the variability of the analytical procedure. The variance of the observations or the measurements is denoted by:

$$s^2 = \pm(x - \bar{x})^2/ (n - 1)$$

or

$$s^2 = (x_1^2 + x_2^2 + \ldots\ldots + x_n^2 - n\bar{x}^2) / (n - 1)$$

and not by δ^2, hence the denominator is n - 1 and not n. We call s^2 the "observed" variance and δ^2 the true variance. The use of n - 1 instead of n does not affect s^2 much when n is large (if n = 100, it causes a 1 percent difference but for values of n around 6 to 10 the difference is appreciable and it is the use of n - 1 that makes it possible for us to obtain results from as few as 6 measurements).

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4. UNITS USED IN CHEMICAL OCEANOGRAPHY AND MARINE CHEMISTRY

by

Stig H. Fonselius

4.1 Introduction

It is possible to express chemical data in many different units. For chemists these units generally do not cause difficulties but oceanographic data are also used by biologists, physicists, geologists, meteorologists, engineers, lawyers, marine and merchant officers, etc. The units used by chemists are often confusing and difficult to understand. Internationally, the use of certain standard units has been agreed upon.

4.2 Salinities and volumes

Salinity and chlorinity are always expressed in per mille weight unit. This means per kg sea water so that a 35 per mille salinity means 35 g salt per kg of sea water. When analysing other constituents in sea water, the analysed constituent has generally been expressed in weight units and the water in volume units, weight/volume. The litre has been used mostly as the volume unit, giving mg/l. In this case, one must pay attention to temperature and pressure. The definition of 1 litre is "the volume of 1 kg pure water at +4°C and 760 mm atmospheric pressure". It is desirable to use the metric system when possible, therefore, the litre is nowadays replaced by the cubic decimeter, dm^3. At +4°C and 760 mm pressure, 1 litre is equal to 1.000027 dm^3. Only in extremely exact work must this small difference be taken into account. In ordinary analytical work in oceanography, it is at present sufficient to regard 1 kg water = 1 litre water = 1 dm^3 water. In geophysical literature, all three units and their multiples are encountered and it is important to understand that they are all approximately synonymous.

1 ton of water is approximately 1 000 litres and approximately 1 m^3
1 kg of water is approximately 1 litre and approximately 1 dm^3
1 g of water is approximately 1 ml and approximately 1 cm^3
1 mg of water is approximately 1 µl and approximately 1 mm^3

Among weight units the expressions µg or γ may be encountered. Both are equal to 0.001 mg. We may also observe that ton/km^3, mg/m^3 and µg/l express the same concentration. These units are very convenient for the calculations of total amounts of different elements. If we, for example, know the concentration of an element in the water from a certain sea in µg/l, it is easy to calculate the total amount of the element in the sea if we know the volume of the sea in km^3. The value in µg/l is equal to the value in ton/km^3 and we have only to multiply the value by the volume of the sea in order to get the total amount in tons.

4.3 Nutrients

In many cases where elements participate in chemical or biological processes, it may be convenient to use units suitable for quantitative chemical reactions, e.g., molecular and atomic weights. Nutrient salts are preferably expressed in such units. The units encountered in oceanography are mg-atoms/l and µg-at/l. These units are 10^{-3} and 10^{-6} g-atoms/l. The unit g-atom is used in American scientific literature but internationally is generally replaced by the unit mole. In oceanography, the old g-atom is widely used, probably because most oceanographers are not chemists and do not desire to change over to new unit names which may be hard to understand. The mole and g-atom are simply different names for the same expression, the g-atom being limited to atoms while the mole includes molecules.

One mole of a chemical element consists of 6.06×10^{23} atoms. Each element consists of a single kind of atom with a given mass. The lightest atom is the hydrogen atom. It weighs $10^{-23}/6.06$ g. One mole of oxygen atoms weighs exactly 16 g and the atomic weight scale has,

therefore, been arranged so that oxygen has the atomic weight of sixteen. Then the unit for the atomic weight will be $1/6.06 \times 10^{23}$ g. The weight of all the atomic species will then be expressed in this unit.

If we express the weight of one atom in atomic weight units as M, the weight in grammes is $Mx10^{-23}/6.06$ and 1 mole of these atoms weighs $6.06x10^{23}$ $xMx10^{-23}/6.06$ g. Simplifying this expression, only M grammes remain. This means that 1 mole of an element weighs as many grammes as the atomic weight of the element indicates. The atomic weights may be found in tables, textbooks, etc., and it is sufficient to know that one mole of an element is equal to its atomic weight in grammes. For example, 1 mole of hydrogen is about 1 g (1.008), 1 mole of sodium is 23 g, 1 mole of chlorine is 35.5 g, etc. The atoms may form molecules, e.g., NaCl, H_2O, etc. One mole of a species of molecules is the sum of the atomic weights in the molecule = NaCl = 23+35, H_2O = 2x1+16.

As was mentioned above, the equivalent g-atom and g-molecule are used in oceanography. Usually the oceanographers are interested in only one specific element in a molecule and, therefore, use the g-atom (preferably µg-at). When, for example, we analyse phosphorus, we get the result as phosphate (PO_4^{3-}). We are, however, only interested in the P atom in the phosphate and express it as µg-at PO_4-P/l. The atomic weight of phosphorus is 31 which means that every µg-at of P weighs 31 µg (0.031 mg). It is obvious that we get the weight of the P in µg if we multiply the amount in µg-at by 31. It is, therefore, simple to transfer µg-at (and its multiples) to µg by multiplying by the atomic weight, and µg to µg-at by dividing by the atomic weight of the element. We must remember that the µg-at refers only to the P in PO_4 and by no means the whole molecule. In the latter case "µ mole" are used. In order to avoid any confusion on the above described, in oceanography one always writes µg-at PO_4-P or NH_3-N, NO_3-N, NO_2-N, SiO_4-Si, H_2S-S, etc.

4.4 Dissolved gases

When analysing gases dissolved in sea water, care must be taken not to confuse gas molecules (O_2, N_2) with di-atoms and simple mono-atomic gases (Ar, Xe, etc.). The first gases mentioned have, of course, two atoms of the same kind - 1 µg-at of oxygen gas (O_2) means 16 µg and not 32 µg. One mole of a gas will, at NTP, occupy a volume of 22.4 litres. This means that 1 mole of oxygen gas (O_2) will have a volume of 22.4 litres and the weight of it wil be 32 g. One g-atom of oxygen in atomic form also has a volume of 22.4 litres. When we talk about oxygen in the sea, it is always in the form of a diatomic gas when in solution while, in biological reactions, it is mono-atomic.

In biological reactions we often use mg-at and, if we wish to transform these to ml, we have to multiply the value in mg-at with the mole volume. But, in that case, we have to remember that we are transforming the oxygen atoms to a diatomic gas (O_2). The volume has to be divided by two and the factor will therefore be 11.2 instead of 22.4. There must be two mg-at of O to give a volume of 22.4 ml.

For example, oxygen expressed in ml/l is transformed to mg-at/l by dividing by 11.2. Nitrogen values are also divided by 11.2 but all other gases in the sea must be divided by 22.4.

For oxygen, the unit ml/l has been used for a very long time. This unit refers to 0°C and 760 mm pressure (NTP). Many attempts have been made to introduce a more convenient unit but, in international committees, the majority have always voted for retaining the old unit largely due to the fact that, with the introduction of a new unit, all old values would have to be recalculated. In fresh-water chemistry, oxygen has usually been expressed in mg/l. In order to convert mg/l O_2 to ml/l, the value is multiplied by 0.7 and, for conversion in the reverse direction, division is performed. For example, 10 mg/l is equal to 7 ml/l of oxygen.

4.5 Conclusions

We may conclude that, as a rule, the organic matter in the sea is expressed in weight units (mg/l); the major constituents also in weight units; the trace elements, if nutrients, preferably in µg-at, and heavy metals preferably in µg. Gases are expressed in ml/l and may also be expressed in mg-at or µg-at.

5. INTRODUCTION TO HYDROGRAPHICAL LABORATORY WORK
A description of instruments and equipment

by

Stig R. Carlberg

5.1 The burette and pipette according to Knudsen

These instruments were constructed by M. Knudsen especially for chlorinity titrations demanding high accuracy and precision.

5.1.1 The burette

The burette is shown in Figure 1. The most noticeable difference between this burette and a common one is the bulb at the upper end. About half of the burette volume is contained in this bulb. Thus the major length of the burette can be used for a very accurate graduation, while covering only half of the total capacity. This means, on the other hand, that the titrations may not vary more than they can be measured on the graduated part. As a consequence of this, the same burette cannot be used for samples of high as well as low chlorinity. If both types of samples are to be analysed (e.g., samples from estuaries) a second burette has to be used. This second one is shown in Figure 2. The only difference with this one is that it has two graduated zones - above and below the bulb.

The graduation on these burettes is in double millilitres (dml) in order to get a numerical value (of the consumed amount of silver nitrate solution) close to the chlorinity of the sample.

When the burette is filled a small amount of solution is allowed to over-flow. This solution is caught in the small container on top of the burette. The supply bottle with silver nitrate solution is connected to the burette as is shown in Figure 1. Upon inflow when the burette is being filled, the over-flow collected at the top is simultaneously sucked back to the supply bottle again.

5.1.2 The pipette

The pipette is shown in Figure 3. Although originally constructed for the chlorinity titrations, this pipette can be used whenever there is a need for a pipette capable of delivering an exact volume with high reproducibility.

In the figure the stop-cock of the pipette is in the delivery position connecting the bent air tube with the bulb. When filling, the stop-cock is turned 180°. The sample is sucked up so that it extends into the straight tube above the stop-cock. The latter one is then turned 90°, and the sample is secured. By turning the stop-cock another 90° the sample is delivered. It is essential never to let the sample come into the air-tube. If it should happen, it leads to measurement errors and cross contamination between samples.

The burette, as well as the pipette, must be carefully calibrated by weighing delivered volumes, if accurate results are expected. This procedure is described in the Chapter 16.1.1. "Titrimetric determination of chlorinity of sea water".

Finally, a bottle provided with a water lock is shown in Figure 4. This bottle is used for storage of standard sea water (SSW). The water lock prevents a change in the concentration of the SSW by evaporation.

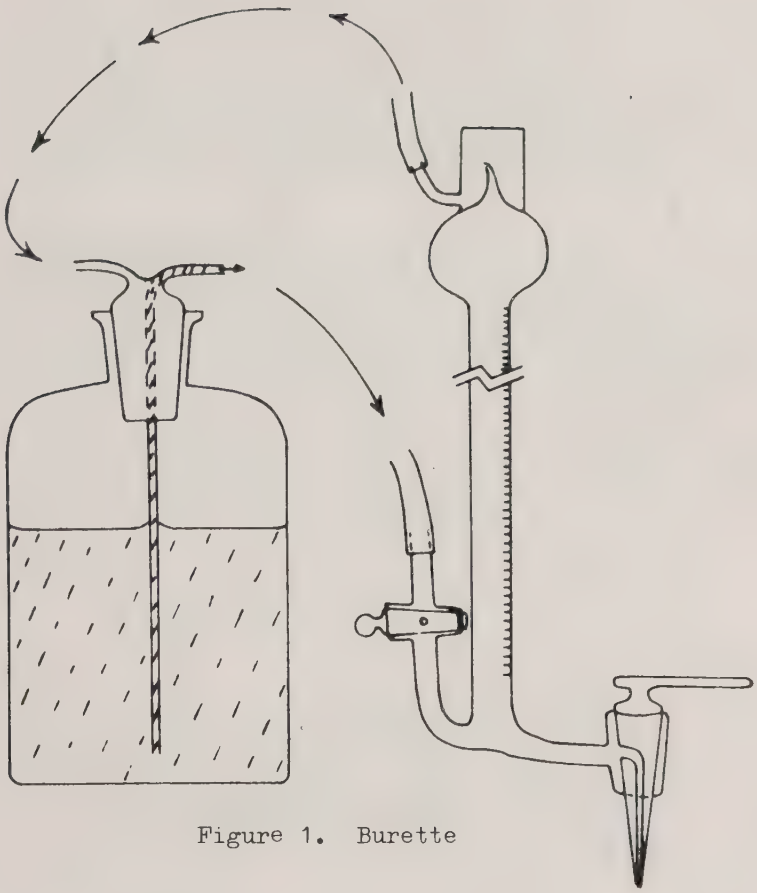


Figure 1. Burette

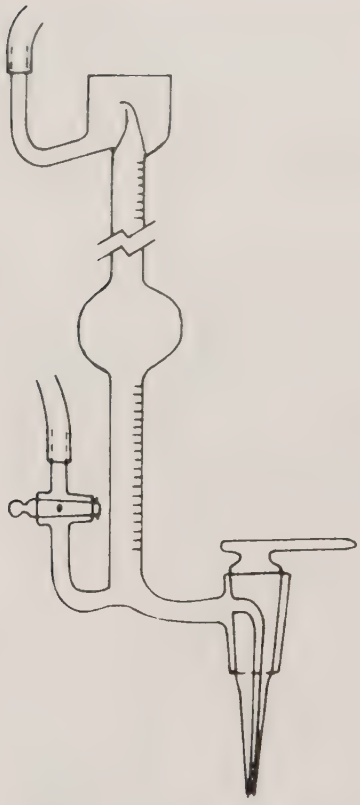


Figure 2. Burette with two graduated zones

Figure 3. Pipette

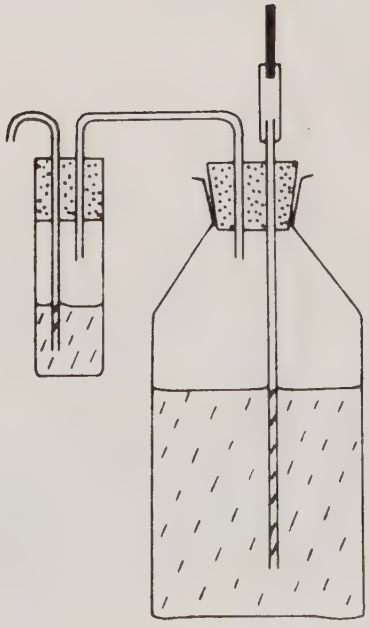


Figure 4. Bottle with water lock

5.2 The density hydrometer

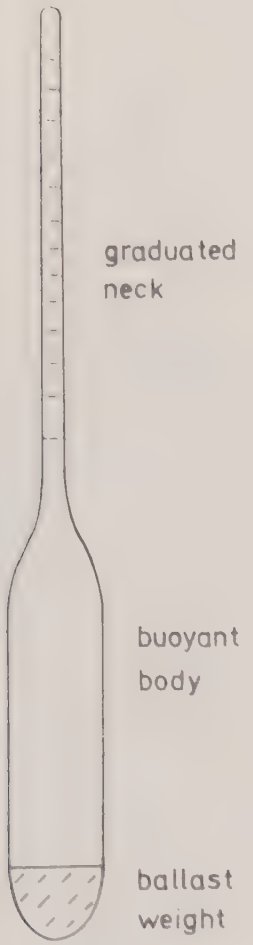


Figure 5. Density hydrometer

The hydrometer is a simple instrument for density (and thus salinity determinations. It consists of a glass bulb with a ballast weight (lead) at the one end and a graduated neck at the other.

The volume of the buoyant body and the weight of the ballast are so chosen and balanced against each other that when the instrument is immersed in the water sample, it floats with a part of the neck above the water surface; the greater the density of the water sample, the longer the part of the neck that will extend above the surface.

One single hydrometer cannot cover the entire range of densities corresponding to salinities from fresh water and up to oceanic water. Therefore the instruments can be purchased in sets, covering the range of interest.

For precise determination, the temperature-dependent volume changes of the buoyant body of the instrument must be compensated for. Originally the instruments were made from "Normalglass 16^{III}" with a coefficient of cubical expansion of:

$$10^{-6}\left\{2\ 316.7\ \frac{t}{100} + 107.1\left(\frac{t}{100}\right)^2\right\}$$

where t = temperature

If another type of glass is used in the instrument, its coefficient of cubical expansion must be known and compensated for.

Every hydrometer is calibrated at a certain temperature (e.g., 17.5°C). If the measurements of samples are carried out at some other temperature, the results must be recalculated, account being taken of the difference between calibration temperature and sample measurement temperature. How this is done is explained in tables supplied with the instruments and also in Knudsen's Hydrographical Tables. In that book a table with correction terms for different glass types is also given. For further explanation of details, see the analytical method in the Chapter 16.1.2 "Determination of sea water salinity using a hydrometer".

5.3 The laboratory salinometer

The laboratory salinometer is a precision instrument for measuring the salinity of sea water samples. Instruments are manufactured and sold by a number of companies in Australia and the United States. The instruments, which have been widely used in oceanographic laboratories so far, are constructed according to one and the same principle. A short description of the working principle is given below together with some typical data for such an instrument.

The instrument is a conductivity-type measuring device which utilizes an inductively-coupled conductivity sensor to establish a conductivity ratio between an unknown sample and a standard of approximately 35 per mille salinity. Actual salinity is then determined by reference to standard tables. A dual-element platinum thermometer and associated circuit sense the temperature of the sample and apply appropriate compensation. For temperature differences up to ± 3°C between the sample and the standard, compensation is fully automatic over the range 0-40°C.

For measuring the conductivity, it would in principle be sufficient to have two metal electrodes in the sample to be tested and to measure the current flowing when voltage is applied. In fact several field instruments for in situ measurements are constructed in this way. For several reasons, this method is not suitable for instruments which must be capable of high precision and high accuracy measurements. Special care must be taken to avoid corrosion, gas bubbles, etc., that influence the effective surface of the electrodes.

For the reasons described above, the laboratory salinometers have so far been equipped with two toroidal coils, rather than electrodes. These coils are inductively coupled by the water sample to be tested with the conductivity.

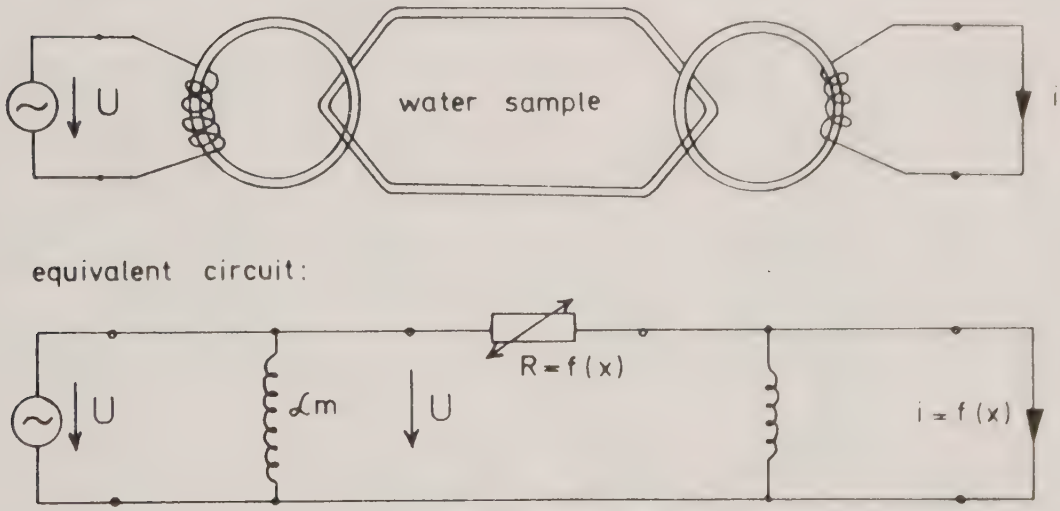


Figure 6. Principle of laboratory salinometer

In one of the coils a constant voltage is generated and the resulting electromagnetic field induces a current in the water. The magnitude of this current depends on how much salt is dissolved in the water sample. The electrical conductivity, and thus the induced current, increases with increasing salt content. In the same way the current in the sample electromagnetically generates a current in the second coil. This secondary current is measured with high accuracy and precision, as it is proportional to the salinity of the sample.

A more detailed description of the inherent parts of the instrument is given in any producer's manual, which is supplied with the instruments.

Some typical specifications for a high precision laboratory salinometer (Bisset Berman, Hytech Marine Products Laboratory Salinometer Model 6220):

Salinity measurement:

- Range	0 to 51 per mille
- Least count	0.0004 per mille
- Accuracy	±0.003 per mille
- Temperature compensation	±0.002 per mille for a variation of ±3°C between sample and standard

Temperature measurement:

- Range	0 to 40°C
- Accuracy	±0.5°C

5.4 The automatic piston burette

This instrument is a very good (more expensive, however) alternative to the common all-glass burette. From the analytical point of view the advantages are: less risk for erratic readings due to the digital read-out, and sometimes increased speed, because one has not to wait for equilibration of the reagent level before taking the reading. In addition to this, the instrument - although some parts are made of glass - is not so delicate as the all-glass burettes. For this latter reason the automatic piston burette may be the best choice for aboard ship analyses.

An example of a commercially available instrument is shown in Figure 7.

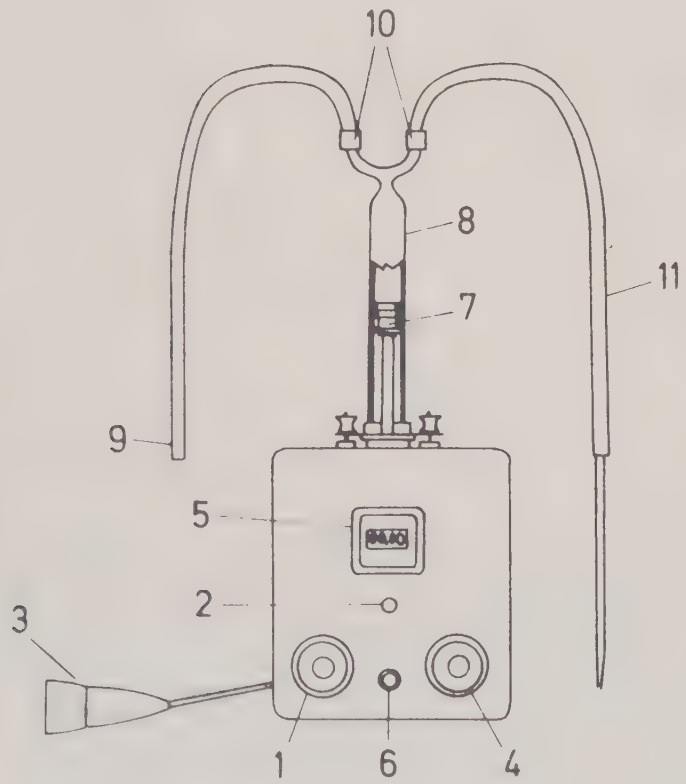


Figure 7. Automatic piston burette
(Reproduced by kind permission of Metrohm AG, Switzerland)

Legend:

(1) Function switch: on/off, continuous flow, stepwise flow
(2) Indicator light
(3) Remote control for delivery of reagent
(4) Speed control for the continuous flow function
(5) Counter for delivered volume of reagent
(6) Push-button for refilling of the burette
(7) Piston
(8) Burette cylinder
(9) Hose from supply bottle
(10) One-way vents
(11) Reagent delivery to the sample

5.5 pH electrodes and potentiometric pH determinations

5.5.1 The principles and a short description of the electrodes.

pH is defined as the negative logarithm of the hydrogen ion activity, a_{H^+}: $pH = -\log a_{H^+}$ is a direct measure of acidity according to the Brönsted definition of acids as substances that are able to donate hydrogen ions, and bases as substances that are able to combine with hydrogen ions: acids $\rightleftharpoons$ base + hydrogen ion.

The principle for the potentiometric determinations is that the potential of the electrode chain consisting of an indicator electrode sensitive to the hydrogen ion activity according to the Nernst equation - and a pH-independent reference electrode, both immersed in the test solution, is measured by means of a suitable galvanometer (pH meter). The measured potential is then compared with the electrode chain potential obtained when a buffer solution is used as test solution, and the actual pH value is found using the formula for "Operational pH":

$$pH(X) = pH(S) + [E(X) - E(S)]/R'T$$

where:

pH(X) and pH(S) = pH of test solution and buffer solution respectively. E(X) and E(S) = electrode chain potential in test solution and buffer respectively. R'T = the Nernst factor (R' = a constant, T = the absolute temperature).

A correctly calibrated pH meter automatically converts the measured potential to give direct reading in pH units.

A detailed description concerning the requirements of the electrodes and their ideal pH response is found in the book by Niels Linnet.

The pH electrode chain most widely used in practice is the glass-calomel electrode chain consisting of a glass electrode with a silver/silver chloride internal reference electrode and a calomel electrode with a salt bridge containing saturated potassium chloride and with a liquid junction via a porous pin. An example is shown schematically in Figure 8, which also indicates the various potential generating elements that contribute to the total electrode chain potential.

The electrode chain may be presented as follows:

$$\underbrace{Ag(s);AgCl(s),Cl^-}_{E_{Ag,AgCl} = E_{o,Ag,AgCl} - R'T \log a_{Cl^-}} \quad \underbrace{Buffer(pH_1) \| glass \| Test\ solution(pH_2)/KCl}_{E_{Membrane} = \frac{R'Ts}{100}(pH_2 - pH_1) + E_{Asymmetry}} \quad \underbrace{E_{liquid\ junction}}$$

$$\underbrace{(sat'd),Hg_2Cl_2(s);Hg(l)}_{E_{Hg,Hg_2Cl_2} = E_{o,Hg,Hg_2Cl_2} - R'T \log a_{Cl^-}}$$

According to the Nernst equation the change in potential per pH unit is 59.2 mV at 25°C. Thus the ideal response of the measuring system should be 59.2 mV/pH. In this case just one buffer sample should be sufficient for standardization of the pH metre. However, as the actual response of electrodes differs from the ideal value to a greater or lesser extent, a two-buffer system is used for standardization. The two buffers are chosen at the two ends of the pH region of interest. In chemical oceanography two buffers of pH 6.865 (25°C) and 8.459 (25°C) are suitable, as they cover the entire normal pH range between estuarine and oceanic waters. With these buffers the actual slope of the sensitivity curve for the electrode system is compensated for.

From intercalibrations of pH measurements it has been experienced that it is not sufficient to use good buffers and careful standardization techniques if the greatest accuracy (approximately ±0.02 pH units) is expected. It is also necessary to prevent gas

exchange between the sample and the atmosphere and to perform the measurements at constant temperature. A detailed description of the necessary equipment and standardization procedure is given in the manual, Chapter 16.2. Furthermore, it is advisable not to store the samples for longer than one hour between sampling and measurement.

5.5.2 Calculation

The measured pH value is not the true pH value of the sampled water. The measured (or apparent) value has to be corrected for the temperature difference between measurement conditions and those at the sample depth in the water. If the sample was obtained from about 500 m or deeper a further correction for the difference in hydrostatic pressure has to be applied.

According to the recalculations and experimental work of Gieskes (1969), the temperature coefficients are only a function of chlorinity and temperature, but not of the pH, considering the limits of errors in the pH measurement itself. Therefore the slight dependence of the temperature coefficient on the temperature and salinity can also be neglected for calculation of the correction member.

The formula for calculation of the $pH_{\underline{in\ situ}}$ is then:

$$pH_{\underline{in\ situ}} = pH_{measured} + 0.0118(t_2 - t_1)$$

where:

t_1 = temperature in situ

t_2 = measurement temperature

This formula is valid for work in areas with water depth not exceeding 500 - 1 000 m (depending on the accuracy desired). The complete correction formula is:

$$pH_{\underline{in\ situ}} = pH_{measured} + 0.0118\ (t_2 - t_1) - \beta \cdot Z;$$

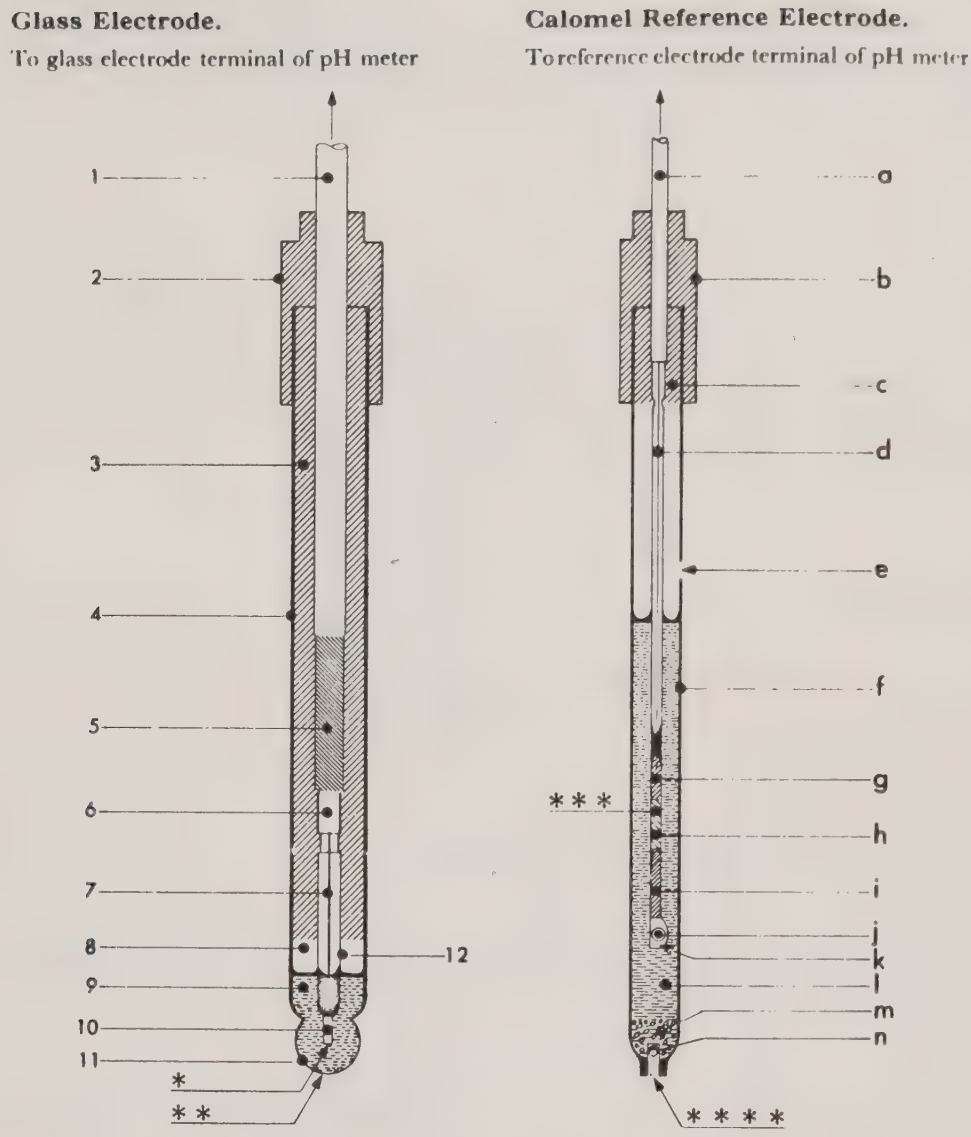
The last term $\beta \cdot Z$ is a correction member for the difference in hydrostatic pressure between the sampling depth and the laboratory at the sea surface. Z is the sampling depth in metres and the β-value is obtained in the table below:

$pH_{measured}$	β	$pH_{measured}$	β
7.5	$35 \cdot 10^{-6}$	8.0	$22 \cdot 10^{-6}$
7.6	31	8.1	21
7.7	28	8.2	20
7.8	25	8.3	20
7.9	23	8.4	20

5.6 The turbidity of water and instrumentation for turbidity determination

5.6.1 Introduction

Any turbidity of water is caused by substances which are not truly dissolved (true solution implies approximate particle size less than 10^{-9} m) but rather present as colloidally dispersed (10^{-7} - 10^{-9} m) or coarsely dispersed (approximate particle size greater than 10^{-7} m). The turbidity cannot be measured by chemical methods.



1 = Shielded lead
2 = Cap
3 = Insulating material
4 = Insulating glass stem
5 = Shield
6 = Insulation
7 = Platinum wire
8 = Air
9 = Chloride-ion-containing buffer solution (pH_1), "Inner Liquid"
10 = Silver wire coated with AgCl, "Internal reference electrode"
11 = Glass membrane, pH sensitive according to the Nernst equation
12 = Glass

a = Lead
b = Cap
c = Insulating material
d = Platinum wire
e = Refill hole
f = Salt bridge
g = Mercury
h = Hg, Hg_2Cl_2, KCl, H_2O paste
i = Cotton wool
j = Contact hole
k = Asbestos
l = Saturated KCl solution
m = KCl crystals
n = Porous ceramic pin

* $E_{Ag, AgCl} = E_{o, Ag, AgCl} - R'T \log a_{Cl} -$

** $E_{Membrane} = \frac{R'T_s}{100} (pH_2 \text{ *}) - pH_1).$

E Asymmetry *) pH_2 = pH of test solution.

*** $E_{Hg, Hg_2Cl_2} = E_{o, Hg, Hg_2Cl_2} - R'T \log a_{Cl} -$

**** $E_{liquid\text{-}junction}$

Figure 8 The pH electrodes

The figure of the pH electrodes and some text on their working principles were taken from the book "pH Measurements in Theory and Practice" by N. Linnet, which was published by Radiometer A/S (Copenhagen), 1970. The material was used with the kind permission of the publishers

The only possibility to obtain an objective measurement of turbidity is to use a specially designed instrument. Most of these instruments are true nephelometers, working on the principle that light, passing through a non-homogenous medium, is scattered (in this case by the particles in the water). This is the Tyndall effect. The turbidity is not related directly to the mass or weight of the particles, but rather to their number, size distribution and surface structure.

5.6.2 Calibration

From the description above it is obvious that the turbidity values cannot be expressed as a weight amount per litre. One has to use arbitrary units by calibrating the instrument with some kind of standard, which can be prepared in a reproducible way. Such a standard is Formazine, a substance that can be prepared by synthesis and reproduced repeatedly to within one percent. For details of preparation see below. The standardization and the analytical results are expressed as FTU - Formazine Turbidity Units, which are equivalent to JTU - Jacson Turbidity Units. Another unit, which will be used in the future, is NTU - Nephelometric Turbidity Units (based on Formazine standard).

Preparation of Formazine standard:

(1) Dissolve 5.000 grammes of reagent grade hydrazine sulphate ($N_2H_4 \cdot H_2SO_4$) in about 400 ml of distilled water.

(2) Dissolve 50.00 grammes of pure hexamethylenetetramine in about 400 ml of distilled water.

(3) Pour the two solutions into a one-litre volumetric flask and dilute to volume with distilled water.

(4) Allow the solution to stand for 48 hours at 20-22°C. During this time the suspension will develop.

The following table gives the relationship between dilutions of the stock solution prepared as described above and FTU's. When diluting the suspension, use "turbidity-free" distilled or demineralized water. Be sure to adequately mix the stock solution prior to removing a portion for dilution.

FTU's	ml of stock suspension diluted to 1 000 ml with "turbidity-free" water
1 000	250
500	125
100	25
50	12.5
10	2.5
5	1.25
1	0.25

5.6.3 Instrumentation

A short description is given below of one of the commercial instruments (Laboratory Turbidimeter Model 2100A from Hach Chemical Company, U.S.A.).

The text describing the Formazine standard and the figure showing the construction of the instrument were reproduced with the kind permission of Hach Chemical Company, U.S.A.

In the instrument a strong light beam is sent upward through a cell containing the sample. As the beam passes through the sample, a certain amount of the light (proportional to the turbidity present) is scattered at a 90-degree angle to the beam and is received by a photomultiplier tube. This light energy is, in turn, converted to an electrical signal which is measured by the panel meter of the instrument. The layout of the instrument is shown in Figure 9.

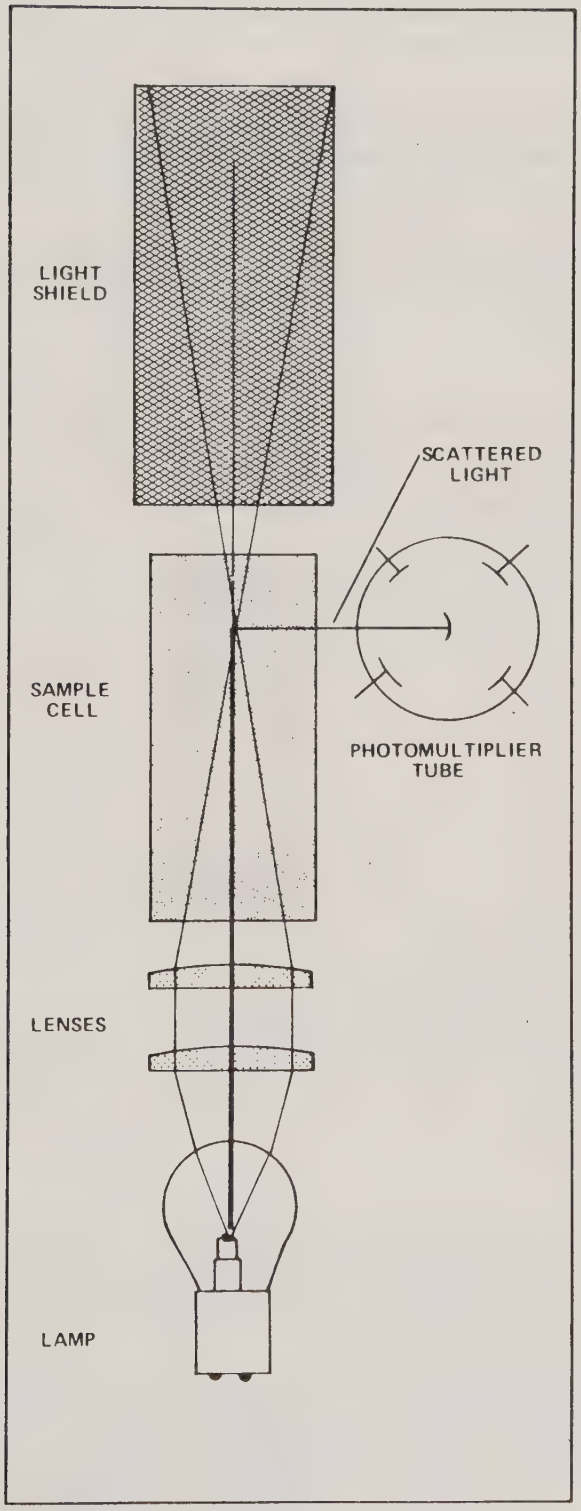


Figure 9. Light path diagram of a turbidimeter

5.7 The spectrophotometer for visual light and its use in analytical work

Many of the chemical methods used in water analyses (such as for nutrients, trace metals) and other fields work according to the principle that the reagents and the substance under investigation, together produce a coloured complex. The intensity of this colour is proportional to the amount of the substance within a certain range of concentrations. The measurement of concentration is thus converted to measurement of colour intensity. The human eye is in this respect not a suitable instrument, as the evaluation of colour intensity is highly subjective. In contrast to this, the spectrophotometer provides a very accurate means for measuring these colour intensities.

In the spectrophotometer a light beam of known intensity is directed toward the sample. A certain part of the light energy is absorbed in the sample and the rest is transmitted. The amount of light energy that is absorbed in the sample is proportional to the intensity of the colour of the sample, and thus the concentration of the sample. This is true if other influencing factors are kept constant.

A further detailed description of the photometric techniques and the instrumentation used is given in the Chapter I:10 "Heavy metal analysis by colorimetric methods".

5.8 References

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6. INTRODUCTION TO ANALYSES OF OXYGEN DEMAND, ORGANIC CARBON, NITROGEN AND SUSPENDED MATTER: MEANS AND GOALS FOR THE RESEARCH

by

Håkan Björndal

(Permanganate method written by Stig R. Carlberg)

6.1 Collection of samples

Methods of collection and analysis must be standardized if the results obtained by different laboratories are to be compared. The "Standard Methods for Examination of Water and Waste Water" describe in detail methods of sampling, analysing and the precautions which have to be taken into consideration. The time between sampling and analysis could in many cases be the critical factor – the shorter the time interval, the more reliable the results. The allowed time is entirely dependent on the character of the sample. A drinking water sample may ordinarily be held for a much longer period than a waste water sample. It depends on the microbiologic activity, which may change the content appreciably. Colour, odour and turbidity may also change during storage. It is not possible to dictate general rules for preventing changes that may occur in a sample bottle. Much time and trouble can often be saved if the best technique for sampling and storage is discussed between the laboratory analyst and the person collecting the samples.

Another problem is how to get representative samples. If the waste water has a constant character, which it seldom has, only a grab sample is necessary. Normally both the composition and the rate of flow vary considerably, under which conditions the collection of a representative sample is difficult. To make an estimation possible the average concentration of pollutants has to be determined in an 8–24 h composite sample. Composite samples are prepared either by a series of grab samples taken at certain time intervals, or from an automatic sampler which also measures the flow rate and takes samples proportional to the flow rate.

6.2 Determination of oxygen demand

The sanitary quality of a water may be obtained by measuring the oxygen concentration. Surface water of good quality should be nearly saturated with dissolved oxygen (DO). However, saturated water may or may not be polluted, but it is not likely to be polluted with high concentrations of oxidizable compounds. The presence of oxidizable matter will decrease the oxygen content due to the microbiological activity. Such compounds have, for their degradation, a certain demand of oxygen which also has been utilized as a method for their quantification. This method, the biochemical oxygen demand (BOD), is today probably the most important method used in describing the degree of pollution of a water supply. The standard method is somewhat tedious. Thus, other oxygen demand methods were developed, namely the chemical oxygen demand (COD), which is much quicker, and the recently developed method for determining the total oxygen demand (TOD).

6.2.1 Biochemical oxygen demand (BOD)

The biochemical oxygen demand (BOD) of waste water is the amount of oxygen required by micro-organisms to stabilize the degradable substances in a water. The oxygen is not only required for the degradation of organic matter, but also for the oxidation of certain inorganic nitrogen compounds and certain reducing substances.

The method involves diluting certain portions of the waste water sample with oxygen-saturated water, seeded with a micro-organism culture. The dissolved oxygen is analysed, both at the start and after a time of incubation, usually five or seven days, at 20°C. The dissolved oxygen content in the bottle is measured either by the Winkler titration with sodium thiosulphate or by using an oxygen-sensitive membrane electrode.

The accepted definition of BOD is a result of research over a century. The principle is relatively simple but the evaluation and instrumentation are still quite complex. Since its introduction, the method has been elaborated to improve its accuracy as an analytical tool. One of the main disadvantages with the dilution method is the step of dilution. By diluting the waste water sample with water, the concentration of the sample contents will naturally be less than in the original waste water. This can result in misleading values since also toxic substances like heavy metals or certain organic compounds will be diluted, thus not affecting the micro-organism population which they probably could in the undiluted water.

The manometric or respirometric method overcomes this disadvantage, since the waste water sample contains a much higher concentration of compounds than the dilution method permits. With this method it is possible to study the rate of oxidation and to correlate this with nutrient addition, waste concentration and toxicity, which is valuable in determining the need for further treatment of the waste water.

The manometric method is based on direct measurement of oxygen consumption by the sample under air or oxygen atmosphere in a closed system. The oxygen uptake is determined at any given time by measuring the decrease in gas pressure. By plotting the oxygen consumption versus time, the ultimate BOD-value can be obtained. Some of the variables and precautions to be considered in these tests will be discussed in more detail later (Chapter 17.2.2). The general biochemical reaction:

$$\text{Organic matter} + \text{DO} \xrightarrow{\text{Micro-organisms}} CO_2 + \text{new cells}$$

is an oversimplified equation of the extremely complex reactions taking place. In order to reproduce, the micro-organisms require nutrients and in a mixed micro-organism population only those successful in the competition for food survive. The food can either be derived from the waste or from dead bacteria cells. The metabolism of the bacteria is chiefly performed by certain protozoa. Besides bacteria and protozoa the mixed micro-organism population usually also contains fungi.

One of the main problems in measuring the biochemical oxygen demand is the oxidation of nitrogen compounds, which of course also consume oxygen and result in a higher value for the BOD. The reaction is called nitrification and is performed by two types of bacteria, namely the Nitroso- and Nitro-bacteria:

$$NH_3 + \text{DO} \xrightarrow[\text{bacteria}]{\text{Nitroso}} NO_2 + \text{DO} \xrightarrow[\text{bacteria}]{\text{Nitro}} NO_3$$

The growth of these bacteria starts after that of the micro-organism responsible for the degradation of the organic content. With a low population of nitrifying bacteria in the seed material used, nitrification will not usually be obtained until several days after the standard incubation period. At present there is no standard method that prevents nitrification in the BOD-test.

Nitrogen, together with phosphorus, is required as nutrient for the growth of micro-organisms in the metabolism of organic compounds. These nutrients are, in the BOD-test, provided as inorganic salts in a phosphate buffer. The necessary amount can be theoretically calculated from the biodegradability of the sample content. Usually a BOD:N:P ratio of 100:5:1 is necessary for the determination. The trace elements required for microbiological growth are generally added.

BOD is used as a parameter in process design and loading calculations as well as a measure of treatment plant efficiency and operation. In this respect the BOD-test is still one of the most important analytical methods.

6.2.2 Chemical oxygen demand (COD)

The chemical oxygen demand (COD) determination provides a measure of the oxygen required for the oxidation of organic matter by a strong chemical oxidant. It is based on the principle that most organic compounds are oxidized to carbon dioxide and water by strong oxidizing agents under acid conditions.

The first chemical oxygen demand test was developed by Forchamer in 1849 using potassium permanganate as the chemical oxidant. Today the dichromate reflux method is usually selected for the chemical oxygen demand determination because it is easy to handle and has an applicability for a wide range of compounds.

6.2.2.1 The dichromate method

A sample is refluxed for a fixed time with known amounts of potassium dichromate and sulphuric acid, and the excess dichromate is titrated with ferrous ammonium sulphate. In most cases, the amount of oxidizable organic compounds is proportional to the potassium dichromate consumed.

Many organic chemicals are not directly oxidizable. This group includes straight-chain aliphatic compounds, aromatic hydrocarbons, and pyridine which are not oxidized to completeness. The result is anyway more reliable than that obtained with the permanganate method.

Addition of silver sulphate to the reaction mixture will, however, catalyze the oxidation of the straight-chain alcohols and acids, although it has no effect on the oxidation of aromatic hydrocarbons.

If the sample contains significant levels of chlorides, bromides or iodides, these will be precipitated by the silver ions, forming precipitates which are oxidized only to some extent by the dichromate. Any oxidation of these halogen compounds will of course give erratic (too high) results. The best way to overcome these disturbances may be to add mercuric sulphate to the sample prior to the addition of the silver sulphate. The halogen ions (chloride is normally the dominating one) are bound to the mercuric ions, forming soluble mercuric complexes. This is, however, not the best solution from the pollution point of view, as laboratories are then potential polluters with the mercury discharge originating from for example COD analyses and sample preservation.

The manual procedure for determining chemical oxygen demand can be automated by using suitable equipment, available from instrument suppliers.

The chemical oxygen demand is an important, rapidly measured parameter for surface and waste water studies and control of the waste treatment plant efficiency. It is especially useful in characterization of waste waters containing toxic compounds, since no reliable value of biochemical oxygen demand can be obtained in such waters.

As the oxidation takes place under severe conditions, the process is more complete than is that of biological oxidation. Thus the COD values (as mg/l O_2) will be higher than the BOD values.

6.2.2.2 The permanganate method

This method is the older of the two oxidative procedures and has in the European countries been applied for decades. As a result of this, great amounts of data have been collected for a number of water sources. In such a situation, it is not easy just to switch over to a newer method as that of dichromate for instance, because the results from the two methods are not always directly comparable. Therefore, if a change of methods is wished, it is recommended to carry out parallel analyses for some time, in order to find out

how big the differences are on the particular water(s) in question. If, however, the intention is to study such investigations in an area, one may choose the method that seems to give the most representative results, or is the most suitable with regard to practical working considerations, etc.

If the dichromate method is the most suitable for analyses of waste water and (severely) polluted surface waters, the permanganate method may be the best choice for unpolluted waters and probably the only possible one for sea waters.

In the dichromate method, the water sample is oxidized after addition of acid and oxidant, by heating for a short time in a boiling water bath. The oxidation effect may be increased by heating the sample to boiling on a Bunsen burner or heating plate. The values from the two ways of heating are not directly convertible into one another, however.

The permanganate method is applicable down to about [illegible] mg/l of consumed $KMnO_4$ (in this analysis expressions for COD - [illegible] mg/l O), depending on the circumstances. For the dichromate method the limits stated in Standard Methods (see references) are 50 mg/l O for the strong solution and 5 mg/l O using the weaker solution. It is still possible to analyse below this limit, but the values become uncertain. Use of the weaker oxidation solution has also been reported to give high and very variable blank values (Bottura and Hochmüller, 1959).

The permanganate method will of course also oxidize the halogen compounds - mainly chloride in natural waters - to free gas. This difficulty can be overcome by carrying out the oxidation in alkaline solution, and then acidifying just in the final titration step when the remaining excess of oxidant is to be determined. In this way sea water (even the highly saline Arctic waters) can be analysed. However, one has to remember that results from acidic and alkaline oxidations are not directly comparable.

2.1.3 Total oxygen demand

One of the most recent methods for waste water analysis is the total oxygen demand (TOD), which is based on the quantitative measurement of the amount of oxygen necessary to combust the constituents in a water sample. The method does not differentiate between organic and inorganic compounds.

The analysis is performed through injection of a small sample volume (20 µl) onto the surface of a platinum catalyst in a combustion chamber at a temperature of 900°C. A constant flow of purified nitrogen gas, containing a fixed concentration of oxygen is flushed through this chamber. The amount of oxygen required for the catalytic oxidation of the impurities is measured as a decrease of oxygen concentration in the nitrogen gas.

Factors like the oxygen demand of hydrogen, nitrogen, and sulphur, as well as the carbon monoxide - carbon dioxide ratio in the combustion process have to be considered before this method can replace other standard techniques.

2.1.4 Total organic carbon (TOC)

The parameter, total organic carbon, is not actually a measure of the oxygen demand of a certain sample, but a good estimate of the oxygen demand can be calculated from the total organic carbon value. Total organic carbon (TOC) is a parameter which is easy to define, instrumentally difficult to determine, and at present somewhat confusing to use or interpret. The total organic carbon content of water would be the sum of the dissolved organic carbon and suspended organic carbon components. The dissolved organic carbon (DOC) component is defined as that portion of the total organic carbon in water which passes through a 0.45 micron filter, while the suspended organic carbon component is that portion which remains [illegible]. The TOC is usually determined by instrumental methods. The two most common instruments are produced by Beckman, Inc. and Dohrmann Instrument Corporation. The Beckman apparatus works according to the oxidation-detection scheme mentioned below, while the Dohrmann model works with a wet oxidation. The advantages

of the Beckman unit are that both organic and inorganic carbon analyses can be performed on less than 1 ml of sample, and that a water sample can be analysed for volatile organic constituents. The advantages of the Oceanography International unit are that a greater precision and accuracy can be obtained, especially at low carbon concentrations, since a 10 ml water sample is used, and that both fresh and saline waters can be analysed. The Beckman unit is not made for analysis of saline waste waters. Another disadvantage with the Beckman apparatus is that the injection system does not allow for any suspended matter in the sample, which may, if present, clog the system. The tests are therefore mostly performed on filtered or centrifuged samples and the values obtained are consequently to be assigned as dissolved carbon. When measuring only the organic carbon content, the inorganic part, represented by carbonate-bicarbonate, must be removed. This is easily done by flushing the sample with nitrogen after acidification.

The Total Carbon Analyser (Beckman Inc.) measures the total organic carbon in a couple of minutes in the range of 1-150 mg/l. The sample (20-50 µl) is injected into a combustion chamber at 950-970°C under a constant flow of oxygen. The impurities are oxidized with the aid of a catalyst, composed of cobalt-impregnated asbestos. The produced CO_2 and water are flushed out of the chamber, where the water is condensed. The CO_2 is then flushed into an infrared CO_2 analyser and the concentration of CO_2 is recorded. Another method for measuring the subsequently produced CO_2 is to reduce the CO_2 and subsequently determine the produced methane with a flame ionization detector.

6.4 BOD, COD, TOD or TOC?

Earlier the measurement of pollution and oxidation process loadings were mostly carried out by wet laboratory methods, such as biochemical oxygen demand and chemical oxygen demand. With recent advances in technology, more accurate methods have been developed based on instrumentation. These include the total oxygen demand and the total organic carbon.

How can these four test procedures for measuring pollution be compared and is there any one that is most meaningful? The biochemical oxygen demand test has been subjected to criticism, as to its value as an accurate measure of the overall pollution load of any total water system.

The biochemical oxygen demand is and was only intended to be a general, qualitative or semi-quantitative index of organic compounds which would rapidly degrade in a short period of time. Today, however, the value of the biochemical oxygen demand has often incorrectly been used as the equivalent to organic load in water and waste waters, regardless of the presence of undegradable organic compounds or of local changes in micro-organism populations. Consequently, the biochemical oxygen demand test should be restricted to those types of waters and measurements for which it was intended. These include the process design of treatment plants, efficiency of treatment plants and the capacity of a receiving water to assimilate organic effluents. The determinations should only be performed if toxic materials are absent. The biochemical oxygen demand test is further dependent on several factors. Even if the acclimation of seed material is standardized much is still unknown about the kinds of bacteria that remain. Different bacteria metabolize specific organic compounds at different rates, which probably will result in marked differences in individual tests when more than one seed material is used. Other factors, like the presence of oxygen or toxic materials in the test bottles, inadequate temperature or dilution, poor adaptation or nitrogen oxidation, will greatly influence the oxygen comsumption curve obtained.

Because of the limitations of the biochemical oxygen demand test and especially the slow biodegradation of many organic substances, the chemical oxygen demand test was developed for the determination of the ultimate biochemical oxygen demand. The main disadvantages of the chemical oxygen demand test are that oxygen is also consumed by the oxidation of inorganic substances, such as reduced metal ions, nitrites, sulphides, thiosulfates, etc., and that certain organic compounds, such as aromatic hydrocarbons, escape analysis. The test is a poor measure of organic loadings unless inorganic chemical constituents are determined

simultaneously. The main usage that the method has today is as a controlling device for the function of treatment plants. Because of the rapidity of the determination it will be possible to obtain data, which could regulate the treatment plant toward optimum efficiency. The test will not provide any data on the rate at which organic compounds could be oxidized by microbial activity.

The total organic carbon test determines, as mentioned before, the total organic carbon content of the waste water sample. The possibility of measuring a broad spectrum of substances affords an assessment of the oxygen-demanding load of the total organic material in a water supply. For the purpose of utilizing the total organic carbon value as an estimate for the biochemical oxygen demand and chemical oxygen demand, several attempts have been made to establish the relationships. For certain types of waste waters a reasonably good correlation has been obtained, provided that the composition of the waste water varies only slightly. Like the chemical oxygen demand test the total organic carbon determination offers no indication on the rate of degradation. A certain portion of the organic material, depending on the type of waste water, is in fact degradable only over a long period of time - up to months or years - and thus not detectable by the biochemical oxygen demand test either. Therefore, the question remains of how to differentiate the degradable matter from the non-degradable materials. The only possibility seems to fall back on the biochemical oxygen demand test in combination with either the chemical oxygen demand test or the total organic carbon test.

The total oxygen demand is, as already mentioned, a recently developed method for determination of carbon in aqueous media. Since the method is new only few data are available on the applicability. Analysis of domestic waste water by this method has shown that the total oxygen demand values were consistently higher than the chemical oxygen demand values and thus higher than the biochemical oxygen demand values. This is probably due to the extreme conditions of oxidation.

The main advantage with both the total organic carbon and the total oxygen demand measurements is that they only require about five minutes - this is often desirable in identification and quantification of pollution. However, many laboratories or authorities might, at present, find the investment too high. It appears, therefore, with consideration to the limitation just mentioned, that the established standard tests for biochemical and chemical oxygen demand will continue at least until a less expensive and fully understandable carbon test is developed.

6.5 Determination of water solids (suspended matter)

The tests for the various types of solids or residues do not determine chemical substances, but rather classes of compounds which have similarities in physical properties. Therefore, the tests are basically empirical and the constituents of each form of residue are defined largely by the test procedure. Accordingly, a close attention is required to such procedural details as time for drying and ignition, temperature for drying and ignition, and filter characteristics in order to obtain reproducible or comparable results.

The term waste water solids is so obscure, that some discussion of the definitions, assumptions and relationships is necessary for proper understanding. Waste water solids are generally classified as total, depositable, suspended, colloidal and dissolved.

Total solids represent the material left in the bottle after evaporation and subsequent drying of a waste water sample in an oven at a fixed temperature. Total solids include "nonfiltrable" and "filtrable" residues. In the past, the terms "suspended" and "dissolved" residues corresponded to nonfiltrable and filtrable residues, respectively. The latter are more precise designations even though somewhat indistinct in definition. The separation is dependent on a number of factors, some of which can only be controlled with difficulty. The main variables involved are the chemical and physical nature of the material in suspension,

the pore size of the filter used, the area and thickness of the filter, and the amount of and physical state of the material remaining on the filter. If colloidal solids are present in the sample, a filter with a given porosity will retain more of the nonfiltrable solids than justified by the porosity due to the clogging effect of the colloids.

Colloidal solids are an entirely different matter. They can technically be defined as particles in the size range from 1 to 500 millimicrons representing an intermediate fraction between nonfiltrable and filtrable solids. Standard methods of determination are not as readily available as for the other solid fractions.

Solids of any of these physically-defined fractions can be further divided into organic and inorganic or hydrophilic and hydrophobic fractions. To obtain the inorganic part, and consequently also the organic part, the dry solid is ignited at 550°C where the residue obtained represents the inorganic part. The differentiation between hydrophilic and hydrophobic parts is easily performed by extraction methods.

The amount of suspended solids removed by a filter depends on the porosity of the filter. A number of the common paper filters used in water analysis are suitable for this purpose. However, due to errors in weighing caused by the hygroscopic paper filter, it has been replaced by the glass-fibre filter to a large extent. To avoid some of the clogging effect obtained by the colloids present in the sample, a filter with a suitable porosity has to be chosen. Samples containing chiefly organic suspended solids are separated by a filter with a pore size of 10 µm, while determination of inorganic matter in suspension is performed with a 1 µm pore size filter.

The drying temperature is of importance for the results, since factors like weight losses due to volatilization of organic matter, water of crystallization, gases from heat-induced chemical decomposition, as well as weight gains due to oxidation, are dependent on the temperature and the period of heating. Usually the filters are dried at approximately 105°C for a period of 1 hour. The determination of total solids by evaporation is impaired by the same type of deficiency.

The analyses may be of limited value for estimating the effect of an effluent on a receiving water, but it may be useful as a control in plant operations, e.g., in measuring the efficiency of sedimentation ponds or in checking the sludge content and the sludge production in a biological treatment plant.

In samples such as river and lake sediments, sludge separated from waste water treatment processes or sludge cakes from dewatering processes like vacuum filtration or centrifugation, it is difficult and usually not necessary to distinguish between filtrable and nonfiltrable solids. Generally, only the total solids on evaporation and its fixed and volatile fractions are determined.

6.6 Determination of nitrogen compounds

Nitrogen occurs in natural and waste waters in a number of different forms. Although molecular nitrogen is a major constituent of the earth's atmosphere, the nitrogen molecule is chemically extremely inert and is not a suitable source of the element for most living organisms. Almost all higher plants, animals, and micro-organisms depend on combined nitrogen for their nutrition. Combined nitrogen, like in ammonia, nitrates and organic compounds, is relatively unusual in soil and water, and its concentration often becomes a limiting factor in the development of living organisms.

The cyclic transformation of nitrogenous compounds, including mineralization of nitrogenous organic matter, is therefore of great importance in the total turnover of this element in the biosphere.

Municipal, industrial, and agricultural waste water disposal has resulted in an increase of nutrients, like nitrogen and phosphorus compounds. This activity may cause an excessive algae and weed growth and contribute to a depletion of oxygen near the bottom caused by the degradation of dead organic matter. This situation, the eutrophication, will usually remain for a long time even if the source of nutrients is cut off.

Nitrogen in the form of ammonia is present in many surface and ground waters. Since ammonia is a product of microbial activity, ammonification, the occurrence of ammonia in surface supplies is sometimes accepted as the chemical evidence of sanitary pollution. The presence of ammonia in ground waters is quite general as a result of natural degradation processes.

Nitrate represents the highest oxidized form in the nitrogen cycle. It normally reaches important concentrations in the final stages of biological oxidation. Generally, it occurs in trace quantities in surface waters, but may reach high levels in certain ground waters.

Nitrite represents an intermediate form in the nitrogen cycle. It may be present in waters as a result of the biological degradation of proteins. When correlating the nitrite concentration with the concentrations of other nitrogen forms, trace amounts of nitrite may indicate organic pollution. Nitrite may also be produced in water treatment plants or in the distribution system through the action of micro-organisms on ammonia, which may have been fed in the combined residual chlorination of water. Another possible source of nitrite entering the water system is its use as corrosion inhibitor in industrial process waters.

The organic nitrogen content of a water is composed, to various extent, of amino acids, polypeptides and proteins, which are all products of biological activity. An increase in the organic nitrogen content in a given water system may often be related to a sewage or an industrial pollution.

To estimate the effect of a pollution load on a receiving water, especially regarding eutrophication, all nitrogen forms have to be determined. The nitrogen content is usually reported as the sum of organic and inorganic nitrogen. The analysis is performed mainly through two different types of analytical systems. The different nitrogen forms are measured separately, where the ammonia is distilled off and analysed after absorption in acid, the nitrate is reduced to nitrite and the sum of nitrate - nitrite - nitrogen is analysed colorimetrically after reaction with a highly coloured azo dye. The organic nitrogen is transferred to ammonia, through a digestion procedure, and analysed accordingly. The other system includes an oxidation of the total nitrogen content (with potassium persulphate) to nitrate, which in turn is reduced to nitrite.

The methods used for nitrogen analysis are considered as the best according to the present state in analytical chemistry. There are of course many sources of errors and probably the most common is the loss of nitrogen in gaseous form due to reactions between organic and inorganic nitrogen forms.

6.7 References

This introduction is partly based on the following literature, which is recommended for further studies:

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7. PRETREATMENT OF SAMPLES (ORGANIC MATTER, SEDIMENTS AND WATER) FOR SUBSEQUENT DETERMINATION OF HEAVY METALS BY ATOMIC ABSORPTION SPECTROSCOPY

by

Göran Lithner

7.1 Introduction

Trace metal analysis is a necessary tool in water protection due to the environmental hazards connected with heavy metals. Most of these materials are more or less toxic to organisms and as elements they survive all self-purification processes in a water body. Increased supply of these pollutants to the water soon leads to accumulation in the ecosystem and a change in the natural trace metal balance. Thus, as a consequence of long-term effects, an enrichment of metals, introduced by pollution, takes place in sediment and biota. By analysing these components of the ecosystem it is possible to trace heavy metal pollution and to some extent evaluate the biological effects.

The sediments were used as indicators of the trophy of lakes by Züllig (1956), who characterized sediments as "waste depots in the metabolism of water bodies". The trace metal content of river sediments has later been used by Hellmann (1970) and others for characterization of sediments with respect to cultural influence. Abnormally high contents of trace metals in sediment may also arise from mineralization of ore bodies, a fact often used in geochemical prospecting (Brotzen, 1966).

The incorporation and accumulation of trace metals in sediments occur to a great extent by adsorption to sedimenting particulate material (e.g., clays, humus colloids, hydratized iron oxides and planktonic organisms). In the sediments, trace metals exist partly as a geological background in minerals (e.g., clays, micas, feld spars) and partly fixed to particulate material by adsorption or to complexing organic compounds. Hydratized oxides of iron and manganese have been suggested by Jenne (1968) to be important sorbents for trace metals in sediments as well as soils.

Of all methods available for trace metal analysis, some at least can, under favourable conditions, be used for direct measurement on solid samples (e.g., emission spectroscopy, X-ray fluorescence and neutron activation analysis). Most analytical methods, however, presuppose liquid samples. It is thus common practice to use some kind of pretreatment, to bring the sample into solution before analysis. Digestion of biological samples and sediments is thus a necessary prerequisite when using, for example, atomic absorption spectroscopy and colorimetric methods.

7.2 Choice of pretreatment procedure

The choice of suitable pretreatment procedure is governed not only by the type of the sample (sediment, tissue or water) and the subsequent analytical technique (atomic absorption or colorimetry) but also by the aim of the investigation. The analysis at the real trace level is of course much more critical and sensitive to contamination than the analysis at the pollution level. However, it must be remembered that a clear distinction between trace level and pollution level cannot be made:

(a) evaluation of the pollution level presupposes that the trace level is known;

(b) in many cases it is desirable to confirm even small increases in metal concentration, which may appear within a large part of a recipient; in such a case the pollution level is - from the analytical point of view - equivalent to the trace level;

(c) concerning some elements (e.g., cadmium), the pollution level is - also after a considerable increase of its concentration above the background values - such that the problems are still great regarding analysis and contamination risks.

Water samples, because of their comparatively low content of organic matter and minerals, and the fact that part of the metal content may already be present in solution, are generally the most simple to handle. Consequently, the following discussion is mainly devoted to samples of sediments and organic matter.

One pretreatment technique, which seems to be very promising, is to apply low temperature ashing of a freeze dried sample. This method seems to be applicable to all metals except the very volatile mercury and its divalent compounds. From the ashed sample the metals may then be extracted by dilute acids. This technique is suitable for a following analysis by atomic absorption as well as by colorimetry.

In the low temperature ashing the sample is placed in a small boat of quartz glass which is then put into a glass tube in which the pressure is somewhat reduced. A slow stream of oxygen gas is passed through the tube. By the action of a high-frequency electromagnetic field, the oxygen gas is excited, yielding free oxygen atoms; these atoms oxidize the organic matter very efficiently. Therefore the temperature can normally be kept as low as 100-200°C.

However, the low temperature ashing technique is outside the scope of this chapter and will be dealt with in chapter 20.2.

Because of the investment costs, equipment for low temperature ashing cannot be included in the standard instrumentation of every laboratory. In such cases, one has to apply the different, classical, chemical methods to bring the metals into solution.

The chemical methods are best adapted to analysis with atomic absorption because this technique permits the introduction of finely dispersed colloid organic matter (e.g., fats) which may still remain after the pretreatment. It is therefore questionable if these methods are suitable for colorimetric analysis, which presupposes samples completely free from organic matter. Colorimetric analysis in most cases also needs a considerable pH adjustment before colour development or extraction. To avoid addition of large amounts of buffering chemicals (which contain smaller or greater amounts of heavy metals) it is thus an advantage to attain a complete or almost complete evaporation of digestive acids. This addition of metals via buffering substances may not present an important problem when analysing at the pollution level, but at the trace level such additions must be avoided as they may be of the same order of magnitude - or more - as the original metal content in the sample. Consequently, it is advisable in the practice of colorimetric analysis to choose some other digestion procedure than the chemical one, if this is possible.

The great variety of binding mechanisms which exists in sediments permits a certain degree of fractionation with respect to strengths of the binding forces - practically, it is convenient to separate the loosely bound fraction from the total content. Most methods used for classification of sediments originate from agricultural soil chemistry and Züllig (1956) was a pioneer in using these methods on lake sediments. In soil chemistry several mild extraction methods are used in order to measure the fraction of trace metals which is accessible to vegetation (e.g., ammonium acetate, acetic acid and diluted strong acids). Lehnard *et al.* (1962) applied one of these methods to measure the adsorbed, loosely bound fraction of metals in river sediment. With ammonium chloride (pH 7) it was thus possible to extract 5-10 percent of the total content (measured by digestion in 10 percent HCl) of copper and chromium in river sediments. An alternative mild extraction method is that in current use at the Marine Geological Laboratory in Göteborg; the sediment sample is soaked for 48 hours in diluted HCl at a pH of 2.6.

These methods are of course of great interest when assessing the exchange of metals between sediment and water as well as measuring the availability of these elements to biota. However, interest is mainly focused on the methods which permit more or less complete digestion of sediments, methods which are directly applicable in tracing water pollution. Analysis of organisms on the other hand gives a direct measure of the availability of trace metals to biota.

There is a vast literature concerning methods for digestion of organic and mineral matter. Many of these methods are reviewed in the books by Pinta (1971) and Gorsuch (1970) which contain comprehensive references. Gorsuch, in "The destruction of organic matter", thoroughly examines methods of digestion of organic matter and discusses sources of errors in general and with respect to individual elements. Information is also given about methods to check recovery. Some of the most common digestion methods are also more fully described. Chapter 21.3.1 is mainly based on the work by Gorsuch (1970).

7.3 Subsampling and classical chemical procedures

To start with, it is necessary to have a representative subsample in order to obtain a meaningful result in the final analysis. If the original sample is inherently heterogeneous there is a need for homogenization. Pretreatment by homogenization is also often made to facilitate the digestion of the samples. Most homogenizers contain metal parts, however. To avoid the risk of contamination and/or to get a better homogenate the sample may first be freeze dried and thereafter homogenized in a mortar. For further details of this, see chapter 18, "Field sampling and laboratory pretreatment of biological samples for subsequent analysis of pesticides and heavy metals".

Biological material is normally homogenized under wet conditions. Sediments may either be dried, sieved and ground, or homogenized under wet conditions with the aid of a plastic propeller.

From the homogenate a subsample of adequate size, adapted to the sensitivity of the analysis and the expected concentrations in the sample, is taken. Sample sizes corresponding to at least 5–10 g wet material are often necessary for determination of less abundant trace metals.

If a sample is not treated immediately it may be stored either in dry form or in wet form by freezing, or in a closed (melted) glass ampoule.

Wet sediment samples should never be permitted to stand for long periods at room temperature or in a refrigerator before treatment, as precipitation of iron and manganese oxides takes place very quickly, which leads to an accumulation of these and other metals on the walls of the container.

7.3.1 Digestion of organic matter

A good method should fulfil the following requirements: complete destruction of organic matter and a quantitative recovery of metals.

Organic matter is digested by oxidative treatment. Usually, one of the following three methods is used: (a) wet oxidation, (b) dry oxidation, or (c) fusing oxidation.

7.3.1.1 Wet oxidation

Wet oxidation is normally carried out by digestion of the sample in mixtures of the strong acids (sulphuric acid, nitric acid or perchloric acid). Sulphuric acid is often used together with nitric or perchloric acid which act as primary oxidants. Sulphuric acid serves to make more resistant compounds available for further attack by the primary oxidant. It also increases the boiling point of the mixture. Perchloric acid is an extremely effective oxidant and a mixture of perchloric acid and nitric acid has, according to Gorsuch, less disadvantages than any other combination of acids. The use of perchloric acid is, however, under certain circumstances associated with explosion risks (Gorsuch, 1970; chapter 4c).

Sulphuric acid has some obvious disadvantages in trace metal analysis. Its high boiling point makes it difficult to remove the excess acid, which thereby implies considerable complications in the colorimetric analysis where there is often a need for proper control of pH. Sulphuric acid also causes precipitation of insoluble sulphates, especially in samples rich in alkaline earth metals. This causes coprecipitation of lead which forms an insoluble

sulphate. According to Gorsuch, this complication occurs especially when digesting bone samples. Comparative studies made at the National Swedish Environment Protection Board show that digestion of sediments decreases the yield of lead by as much as 30 percent compared with mixtures void of sulphuric acid.

7.3.1.2 Dry oxidation

Dry oxidation is carried out by ashing the sample in a crucible at high temperature, often 450-550°C. The ash is then dissolved in a suitable acid. This is an often used method which has the obvious advantage of permitting digestion of large samples. On the other hand, the high working temperature and dry conditions used, increase the risk of losses by retention on the walls of the crucible and by volatilization. Retention losses are mainly caused by the formation of complex compounds between metal oxides (especially lead oxide) and silica in the walls of the crucible. Volatilization losses take place for inherently volatile substances, like mercury, or by the formation of volatile chlorides (e.g., Zn, Pb) or by reduction to volatile metals, like cadmium. These losses can be diminished by adding suitable ashing aids that form non-volatile compounds with the metal in question. Such ashing aids vary with the type of metal: sulphuric acid, phosphoric acid or different salts (nitrates or borates) are preferred.

7.3.1.3 Fusing oxidation

Fusing oxidation is often carried out in a closed system (bomb) by digestion of the sample in an oxidative melt of, for example, nitrate, perchlorate or peroxide. The oxidation takes place at a high temperature and is very powerful. Since the addition of considerable amounts of chemicals introduces contamination risks, the purity of the chemicals must be carefully checked.

7.3.2 Digestion of sediments

Most of the above-mentioned methods can also be used for the digestion of sediments, as these procedures, except the digestion of organic matter, also dissolve adsorbed metals, sulphide minerals, hydratized iron and manganese oxides and, to some extent, leach out metals from minerals, predominantly clay minerals. Most of the silicates, however, are resistant to attack. To get a complete measure of the geological background - and this is of course hardly ever needed in pollution studies or monitoring programmes - it is thus necessary to use a stronger treatment with, for example, hydrogen fluoride or digestion in an alkaline melt (carbonate, hydroxide). The above-mentioned bomb method (Bernas, 1968) is advantageous also for the digestion of silicates (as the PTFE coating permits the use of hydrogen fluoride).

7.3.3 Digestion of water

Digestion of water samples is necessary as pretreatment to colorimetric analysis, to avoid interferences from complexing organic matter. Different strong acids are used, e.g., sulphuric, nitric and perchloric acid. A new method by Koroleff for the digestion of water in a closed system is designed for the analysis of total phosphorus after autoclavation with potassium peroxodisulphate. This method has also found some application in metal analysis. Some examples are given in chapter 24 "Heavy metal analysis by colorimetric methods". Reference is also made to American Public Health Association (1971).

7.4 Comparison of methods

Among the above-mentioned methods, wet and dry oxidation are most often practised. As stated, dry oxidation implies considerable risk of loss of metals. On the other hand, this method permits handling of large samples which is an advantage when analysing elements occurring in extremely low concentrations. Wet oxidation takes place at considerably lower temperatures than dry oxidation, and the risk of losses by volatilization is thus much less. The dry methods are less time-consuming than the wet ones. (The introduction of acids in

the wet methods necessitates careful checks with blanks.) The use of wet oxidation normally limits the sample size which can be easily handled and consequently also the accuracy of the analysis.

On the whole, wet oxidation normally seems to have fewer drawbacks than dry oxidation; this is especially true when many elements are to be analysed, as these methods give a good recovery for most metals (if sulphuric acid is avoided). Wet oxidation is normally carried out by boiling in an open system (Kjeldahl flask), thus the risk for losses of extremely volatile elements, e.g., mercury. To avoid this the following procedures can be used.

One method is boiling with reflux. It is here worth mentioning the apparatus described by Gorsuch (1970), chapter 9, which permits close control of the procedure by alternating between reflux and distillation.

Other methods are digestion at lower temperatures; these methods are designed especially for mercury but can also be used for other metals. Thus, for the determination of mercury in blood (Uthe et al., 1970; Skare, 1972) one method operates at 50-60°C with a mixture of sulphuric acid and permanganate. Good results have also been obtained with a mixture of nitric acid and perchloric acid (Skare, 1972) at 70-75°C for quantitative recovery of mercury from blood, urine and fish samples. The sample is homogenized by pretreatment in alkali. As is pointed out, the alkaline homogenate also permits storage for long periods even at room temperature. This method is used at the National Swedish Environment Protection Board and is described in chapter 19 "Digestion methods for determination of heavy metal content in water, sediment and biological samples".

Another new method (Paus, 1972) permitting a rapid and complete digestion of biological material is to heat a mixture of acid and sample in a bomb with a PTFE (Teflon) coating inside. This method, which seems very promising and can also be used for silicates (Bernas, 1968), avoids losses by volatilization.

At the National Swedish Environment Protection Board laboratory two wet oxidation methods are used, among others, which permit determination of mercury and other metals with atomic absorption spectroscopy. These methods work at low temperatures, the one mentioned above with perchloric acid and nitric acid (Skare, 1972) at 70°C, and the other with "aqua regia" at 90°C. They are applied to organisms and sediment samples, respectively. Both methods are simple and permit digestion of many samples at a time. The samples are normally digested under wet conditions, which favours the destruction of complexing matter, at the same time as the sample is introduced in as unaltered a form as possible. "Aqua regia" has some disadvantages. The yield of some metals (e.g., Zn, Cu) is too low in sediments rich in organic matter (ignition loss above 25 percent) due mainly to incomplete destruction of the proteinaceous materials, which are very strong complex binders. The procedure is also hazardous to health because of strong development of toxic gases (chlorine, nitrous oxides). Handling of these gases thus presupposes good air ventilation. Therefore, from health aspects, and to assure a maximum yield of the metals, the procedure with perchloric acid is preferable for the digestion of both biological samples and sediments.

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8. INTRODUCTION TO ANALYSES OF HEAVY METALS

by

Gunnar Ekedahl

8.1 Introduction

The water chemist is faced with a wide variety of problems. These problems include not only the analysis of the water samples, but also the number of samples, the time of sampling, the location of the sampling points and the number and kind of analyses required. The sample may be sea water, fresh water or waste water. The sample may also be sediment or an organism. These latter types of samples must be subject to a digestion procedure prior to analysis. On the other hand, many analyses of a given constituent are subject to considerable errors because of interference by other components. The concentrations of many elements, the minor constituents or trace elements, are so low as to impose special problems. Nowadays, various separation and concentration techniques are available for removing interferences or for concentration prior to analysis. Trace metals for example are frequently adsorbed onto suspended matter. It may be important therefore to remove the particulate matter for separate analysis. Owing to the extremely low concentrations of many trace elements, it is imperative to guard against contamination during sampling, storage and analysis. In addition, heavy metal ions may undergo a number of transformations during sample handling. It is essential to note all the serious problems in sampling, preservation and storage of the samples. Finally, considerable attention must be paid to define the objectives of analysis in relation to the use which will be made of the results. Bearing the above facts in mind, the water chemist has to take part in the design of the sampling programmes as well as in the evaluation of the analytical results.

This chapter deals with the chemical analysis of metals in the aquatic environment. It is the purpose to present current methods in common use, including techniques for sampling, preservation and storage.

8.2 Metals in the water environment

The metals of interest include the alkali metals, the alkaline earths, the transition elements and the heavy (or coinage) metals. Depending on the solution phase in which they are dissolved, metal ions may occur in a number of aquometallic or organometallic complexes. It is of importance to consider the forms of the reacting species and all possible interactions between a metal ion and its aqueous environment, particularly as they may affect analytical procedures. Most metals (except Na, Ca, Mg and Fe, Mn) are frequently found in the aquatic environment in micromolar or trace concentrations. Some of these metals are involved in important inorganic and biochemical reactions in natural water. It has been assumed that the availability or toxicity of metals to biochemical systems is solely dependent on metal concentration. However, this assumption is not quite valid, since availability and toxicity effects of metals are highly dependent on the degree and type of metal complex formation. Some elements occur as simple ions, as chloro-anions or as sulphates. Other elements form hydroxo complexes. Thus, for example, iron can exist as Fe^{3+}, but also as $Fe(OH)^{2+}$, $Fe(OH)_2^{+}$, $Fe(OH)_4^{-}$ and even dimers such as $Fe_2(OH)_2^{4+}$. The main factor affecting the relative concentrations of these complexes is pH. In addition, many metal hydroxides, carbonates, silicates can exist in the colloidal state and remain stable for long periods of time. Soluble, trace metal ions can adsorb and associate with these colloidal species, as well as other inorganic or organic suspended matter. Often, the particulate matter is removed for separate analysis. It is customary to use a 0.45 µm membrane filter and to define the elements passing through as "soluble". (See "Manual" from Environmental Protection Agency in the reference list.) However, this fraction will include colloidal compounds and soluble organic chelates. An important effect of this type of complex formation concerns the optimum conditions for an analysis. Analysis of the particulate matter sometimes requires several hours of digestion in concentrated acid to convert the metals to a soluble and analysable state. A final, important factor affecting the metal ion species is the oxidation/reduction

state of the sample solution. Generally, the fact that two or more oxidation states can exist for the same metal can have a profound effect on the analysis of that metal. Metals in the aquatic environment may be found in one or more of the following forms:

(1) Soluble species

(a) Free hydrate metal ions
(b) Metal complexes with inorganic or organic ligands.

(2) Insoluble particulate matter

(a) Colloidal particulate matter
(b) Metal complexes adsorbed on suspended matter.

It is obvious that the chemical state of the metals have a profound influence on the analytical procedure chosen. This point must be kept in mind when one considers the common techniques for metal analysis. A review of "Minor Elements in Sea Water" is given by Goldberg, (1965), and the chemistry of equilibrium systems is well discussed in a recent book by Stumm and Morgan (1970).

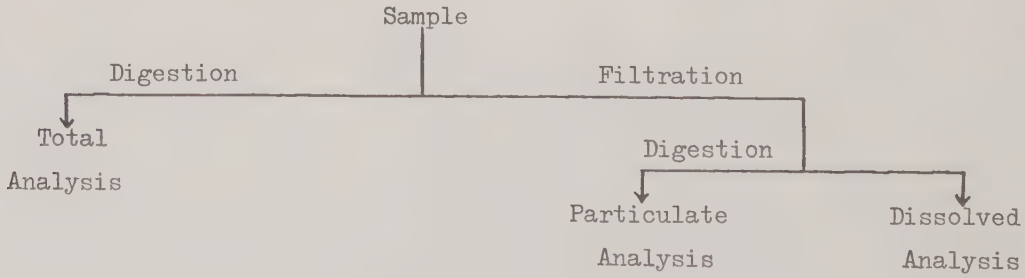
8.3 Sampling, storage and preservation

The sampling procedure must be capable of providing a representative sample. An important part of any sampling programme is the provision of complete information of the source and conditions under which the samples were collected. Owing to the low concentrations of many trace elements, the samples must be guarded against contaminations during collection, storage and analysis. Obviously, metallic samplers should not be used if the water is to be analysed for such metals as copper, lead and zinc. The samples may be filtered and acidified to pH 3.5 with acetic acid, this acidification reduces biological activities in the sample and minimizes precipitation and adsorption on the walls of the sample container. To inhibit the growth of moulds in the acetic acid solution, often a small amount of formaldehyde is added. Nowadays this preservation technique often is replaced by adding concentrated nitric acid to a pH of approximately 2. In many cases the samples are deep-frozen at $-20^{\circ}C$ in polyethylene bottles.

Contamination of the samples with impurities from the reagents or from the storage vessels is frequently a serious problem. High quality reagents are necessary and glass apparatus to be used should be pretreated with diluted nitric acid (1+1). In addition, it is very important that blank determinations are carried out through the whole analytical process.

8.4 Analysis for metals

There are a number of possible variations in procedures for analysis of metal in the water environment.



Digestion techniques are treated in the Chapter 7 "Pretreatment of samples for analysis of heavy metals by atomic absorption spectrometry".

Methods for analysis of metals have changed rapidly during the last three decades. Prior to 1940, most analytical techniques were either gravimetric or volumetric. Modern titrimetric methods are used extensively for analysis of metals, such as complexometric titrations with EDTA and a variety of specific indicators. Perhaps the most well-known examples are the determinations of calcium, magnesium and water hardness. Today this technique can be combined with modern equipment, such as phototitrators.

Photometric methods are probably the most widely used procedures for the determination of trace metals. Since the introduction of reliable and cheap (as compared with other equipments for analysis of trace elements) spectrophotometers, there has been a considerable increase in the use of the spectrophotometric techniques. This increase is also a result of the development of a number of organic reagents such as dithizone, o-phenanthroline, sodium diethyldithiocarbamate and diphenylcarbazide, which form coloured compounds with metal ions in solution (see "Analysis of raw, potable and waste waters" in the literature list). These methods are rapid and often have a high sensitivity. But it must be emphasized that natural water samples are often appreciably turbid owing to the presence of suspended matter. A turbidity correction should be performed, if the samples are analysed directly. But in many cases it is necessary to concentrate the trace elements from a large volume of water before the determination can be made. After concentration, the element may be separated from other elements, interfering in the analytical reaction. The efficiency of the concentration and separation processes must be checked. A number of separation and concentration techniques are available.

The basic procedures are:

(1) Evaporation
(2) Co-precipitation and co-crystallization
(3) Ion-exchange
(4) Partial freezing
(5) Solvent extraction

For a more comprehensive discussion of these procedures the reader is referred to a number of recent publications (Riley, 1965).

Ion-exchange chromatography is a versatile tool for concentration and separation of ions from waters. The ions are selectively eluted from the ion-exchange column and then determined by a suitable analytical method. The selective elution can be improved by using complexing agents. Attempts have been made recently to use ion-exchange membranes. The most interesting aspect of this technique is its applicability for in situ operation.

Solvent extraction is frequently used for metal separation. In order to make the metal-ion extractable, the coordinated water molecules are replaced by a complexing agent. The most used complexing agent is probably dithizone, which forms complexes soluble in chloroform or carbon tetrachloride. This method can be made fairly specific by adjustment of the pH. Since the metal-dithizone complexes have high molar absorptivities, this reagent is suitable not only for separation purposes but also for the final photometric determination.

8.5 Instrumental methods for metal analysis

8.5.1 Absorption spectrophotometry

(a) Molecular absorption spectrophotometry

The most widely used methods for the analysis of metals in water involve the absorption of light by the metals in the form of soluble complexes. This technique has already been mentioned. Although many of these photometric and colorimetric techniques have been used

for many years and may require only rather inexpensive instrumentation, they are in some cases as accurate, precise and sensitive as the most modern methods. Typical sensitivities are about 10^{-6} mole/l. Obviously, this technique is by no means out of date, a great number of new reagents and applications are mentioned in recent review publications (Fishman and Erdman, 1971). These methods are also well-suited for continuous monitoring with automated instruments. A more detailed discussion of these methods is given in the Chapter 10 "Heavy metal analysis by colorimetric methods".

(b) Molecular fluorescence spectrophotometry

This technique offers some advantages over molecular absorption spectrophotometry with respect to selectivity and sensitivity. In this technique a solution of the sample which has been treated with a suitable reagent is irradiated with ultraviolet light. This light irradiation excites the sample complex and the re-emitted radiation has its intensity maximum at a longer wavelength (is of lower frequency) than the existing irradiation. A common fluorogenic agent is 8-hydroxyquinoline.

(c) Atomic absorption spectrophotometry (AAS)

AAS is a relatively new method. It has wide application in the analysis of metals because of its speed, sensitivity, simplicity and ability to analyse complex mixtures without prior separation. In contrast to flame photometry there is little inter-element interference in this technique, because each metal absorbs at a well-defined and relatively narrow wavelength range. More than 60 elements can be readily determined by AAS in the ppm-range with high accuracy and precision. The sensitivity can be vastly increased by different pretreatments of the sample, such as solvent extraction. In addition to concentrating the metal, AAS has a higher sensitivity for many metals in organic solvents than in water. For example, µg/l quantities of Co, Cu, Fe, Pb, Ni and Zn have been determined in saline waters by extracting the metal with ammoniumpyrrolidine dithiocarbamate (APDC) in methylisobutylketone (MIBK). In direct determinations the presence of large amounts of suspended matter can reduce the light absorption. It is also reported that salinity differences between samples and standards can be a source of error. Standards must match the sample solutions in salinity. These errors can be mastered by the use of the "deuterium background technique" which compensates for non-atomic absorption. For determination of mercury a "flameless" technique has been introduced; after a digestion procedure, the mercuric compound is reduced to the free element which is volatilized in a current of air and passed to a silica absorption cell mounted in place of the flame in an otherwise conventional AAS-equipment. The sensitivity can also be vastly increased by use of a number of accessory techniques, such as "the boat technique", "the carbon rod technique" and "the graphite furnace technique" (Willis, 1973). A detailed account of AAS is given in the Chapter 9 "Heavy metal analysis by atomic absorption spectroscopy and flame emission spectroscopy".

(d) Atomatic fluorescence spectrophotometry (AFS)

In AFS the atoms in the flame are excited in exactly the same way as in atomic absorption. However, in this case the emitted light is then measured as the atoms return to their ground state.

AFS offers no increase in selectivity over AAS, but it is possible to increase the sensitivity with this method. AFS is a relatively new technique and has not been much used in water analysis, but modern equipment is available.

8.5.2 Emission spectrometry

Emission spectrometry is based on the measurement of radiation emitted from elements that have been volatilized and excited by a high energy source, normally an electric arc. After recording the spectra, the presence of different emission lines can be used for qualitative identification and the intensity of the lines for quantitative analysis.

This method is not so frequently used; few laboratories have the required instrumentation and the highly trained staff required for the proper operations. In most cases this technique is not sufficiently sensitive, the samples must be preconcentrated by evaporation. However, the method is suitable for analysing a large number of samples and in addition this method can tolerate large quantities of dissolved solids.

8.5.3 Flame emission photometry

This technique is also based on the measurement of radiation emitted from elements, which are excited by spraying a sample solution into a gas flame. The wavelength of the emitted radiation is qualitatively characteristic of the determinant and the intensity depends on the concentration. Normally photoelectric detection is used.

Flame photometry is a useful method for determining the presence and amounts of alkali and alkaline earth metals. With the introduction of the AAS-technique, flame photometry has been practically restricted to the analysis of these metals. For some elements, flame photometry is still to be preferred. A comparison of AAS and flame photometry sensitivities is given by Martin (1968).

8.5.4 Activation analysis

Activation analysis involves irradiation of a sample (usually with thermal neutrons) to induce nuclear transformations. The induced activity is proportional to the amount of the reactive nuclide, and is independent of its chemical state.

Very high sensitivities can be attained by neutron activation analysis. Examples of sensitivities are in µg/l:Zn 0.006, Co 0.0006 and Cu 0.003. However, this technique requires the use of elaborate and expensive equipment not generally available. In addition, elaborate separation processes are often required both prior to and after irradiation. For example, seawater samples must be allowed to "cool" for a suitable period of time after irradiation in order to let the intense activities caused by the short-lived ^{38}Cl and ^{24}Na to die away.

8.5.5 Electrochemical analysis

(a) Polarography

In polarography, special current-voltage curves, called polarograms, are obtained by the use of an instrument, known as the polarograph. From a polarogram both qualitative and quantitative determinations can be made.

Polarography is a useful tool in water analysis. The sensitivity of classical polarography with the dropping mercury electrode is limited to about 10^{-5} mole/l. However, by means of preconcentration techniques the sensitivity can be extended significantly. This method is also suited to analyse mixtures, thus reducing the need for prior separations. One serious drawback is the interference produced by electroactive and surface-active impurities. The use of modern techniques increases the sensitivity, pulse polarography is sensitive in the range 10^{-7} - 10^{-8} mole/l, cathode ray polarography 10^{-7} mole/l and anodic stripping polarography better than 10^{-9} mole/l.

(b) Ion-selective electrodes

These electrodes are basically potentiometric membrane electrodes which operate on a principle similar to the well-known glass electrode for the determination of pH. Electrodes are commercially available for the measurement of Ca^{2+}, Mg^{2+}, Cu^{2+}, Cd^{2+}, Ag^{+} and Pb^{2+}, (Table II) Their major advantages for use in water analysis are their speed and ability to be used for in situ measurements. The detection limits are in the range 10^{-5} - 10^{-6} mole/l. However, it must be remembered that the electrodes are electrochemical sensors measuring activities rather than concentrations. It is important to consider the effects of the ionic strength of the test solution compared with that of the standard solution used for the

the calibration of the instrument. Finally, these electrodes are usually sensitive only to free or unbound metal ions. Recent work on cadmium has shown that only a minor proportion of the total cadmium in fresh water is likely to be free. Perhaps this determination of the free metal ion is more useful, from a biological point of view, than the measurement of the total concentration.

(c) Anodic stripping voltammetry (ASV)

One of the most interesting techniques in metal analysis of trace quantities is anodic stripping voltammetry. This procedure involves two steps, a concentration step and an anodic dissolution process (reverse electrolysis or stripping). In the first step, the concentration step, the metal of interest is reduced by deposition as a metal or by formation of an amalgam with a mercury drop or film. The deposition can be quantitative by separating the entire or a reproducible fraction of the desired component. The stripping process is carried out by a procedure which produces a response proportional to the amount of metal deposited. This technique has been applied to the analysis of a number of metals. The sensitivity is in the range 10^{-7} - 10^{-10} mole/l.

Table I

Sensitivity limits of common analytical procedures
From Mancy, K.H. (1972)

Method of analysis	Sensitivity mole/l
Molecular absorption spectrophotometry	10^{-5} - 10^{-6}
Molecular fluorescence spectrophotometry	10^{-7} - 10^{-8}
Atomic absorption spectrophotometry	10^{-6} - 10^{-7}
Atomic fluorescence spectrophotometry	10^{-7} - 10^{-8}
Optical and emission spectroscopy	10^{-5} - 10^{-6}
Neutron activation analysis	10^{-9} - 10^{-10}
Potentiometry with metal, specific glass or membrane electrodes	10^{-4} - 10^{-5}
Classical polarography	10^{-5} - 10^{-6}
Derivative polarography	10^{-6} - 10^{-7}
Square-wave and linear-sweep voltammetry	10^{-7} - 10^{-8}
Anodic stripping voltammetry with hanging mercury drop electrodes	10^{-7} - 10^{-9}
Anodic stripping voltammetry with thin film or solid electrodes	10^{-9} - 10^{-10}

Table II

Commercially available ion-selective electrodes

From Mancy, K.H., ed. (1971)

Ion	Membrane	Lower Detection Limit	Principal Interferences
H^+	glass	10^{-14} M	None below pH 13
Na^+	glass	10^{-6} M	H^+, Ag^+
K^+	glass	10^{-6} M	H^+, Ag^+, Na^+
NO_3^-	liquid	10^{-5} M	I^-, Br^-
Cl^-	liquid	10^{-5} M	I^-, NO_3^-, Br^-
Ca^{++}	liquid	10^{-5} M	Zn^{++}, Fe^{++}, Pb^{++}, Cu^{++}
Water hardness	liquid	10^{-8} M	Zn^{++}, Fe^{++}, Pb^{++}
F^-	solid	10^{-6} M	OH^-
Cl^-	solid	5×10^{-5} M	$S^=$, I^-, Br^-, CN^-
Br^-	solid	5×10^{-6} M	I^-, $S^=$
I^-	solid	5×10^{-8} M	$S^=$
$S^=$	solid	10^{-17} M	Hg^{++}
CN^-	solid	10^{-6} M	$S^=$, I^-
Ag^+	solid	10^{-17} M	Hg^{++}
Cd^{++}	solid	10^{-7} M	Ag^+, Cu^{++}, Hg^{++}
Pb^{++}	solid	10^{-7} M	Ag^+, Cu^{++}, Hg^{++}
Cu^{++}	solid	10^{-8} M	Ag^+, Hg^{++}, $S^=$
NH_3/NH_4^+	polymeric	10^{-6} M	Volatile amines

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9. HEAVY METAL ANALYSIS BY ATOMIC ABSORPTION SPECTROSCOPY (AAS) AND FLAME EMISSION SPECTROSCOPY (FES)

by

Östen Lindgren

9.1 Introduction

In analysing waters for metal content, the analyst's major concern is the precise, specific determination of many metals in large numbers of samples. The amounts of metal to be determined may range from trace levels to quite high concentrations although most analyses will involve determinations at low or trace levels. Analytical programmes may range from day-to-day monitoring of small industrial systems to large-scale studies of ground waters or extensive surveys of sea water. In many of these programmes, the large number of samples required and the susceptibility of samples to contamination and element loss create special problems for the water analyst. In view of these considerations, it is essential to use an analytical method which is fast, accurate and simple, involves minimal sample preparation, is specific and free from intereferences, can be used for both high and low concentrations of metals and is non-subjective. Atomic absorption and flame emission spectroscopy are two analytical procedures which satisfy these criteria and have become the preferred methods for metal analysis.

9.2 How atomic absorption works

The atomic absorption technique is based on the fact that the atoms of every element can absorb radiation at very narrow wave-length bands which are different for each element. In order to be in a condition to absorb, the atoms must be chemically unbound and in their minimum energy state; in general this condition is achieved by vaporizing the sample in a flame.

Other techniques are also used for this purpose, the aim being mainly to lower detection limits. Mercury which is very easily volatilized, is often vaporized with air after reduction with some stannous salt (cold vapour technique). Heating samples on a carbon rod, in a graphite cuvette (oven) or in a sampling boat can also be used for some metals when very low detection limits are desirable.

The source of radiation is generally a hollow cathode lamp, whose cathode is made of the element being determined and which emits the line spectrum of that element (Figure 1a). As shown in Figure 1b, the sample absorbs only at a certain line (called the resonance line), giving the result shown in Figure 1c. Here, after passing the flame containing the sample vapour, the resonance line has been diminished by the sample absorption, while all other lines are unaffected. The other lines are removed by a monochromator, tuned to select a band of wave-lengths around the resonance line and to reject all others (Figure 1d). The photodetector then sees only the diminished resonance line (Figure 1e). Atomic absorption obeys Beer's Law: concentration of the element in the sample is proportional to the absorbance (optical density, $\log \frac{1}{\text{transmittance}}$) in the flame.

9.2.1 Atomic absorption systems

The first instruments for atomic absorption were designed to measure direct current (DC) and suffered from the disadvantage of measuring light from the flame as well as from the hollow cathode lamp. Many modern commercial instruments utilize the single beam alternating current (AC) system, shown in Figure 2.

Light from the hollow cathode lamp is chopped and then passes through the flame, after which the resonance wave-length is isolated by a monochromator or filter and then falls onto

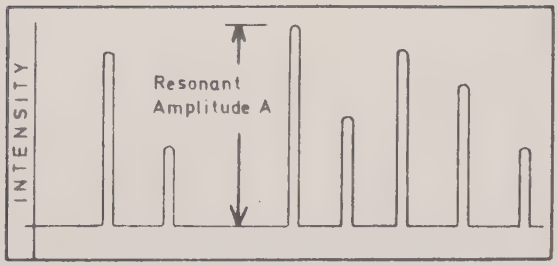

Figure 1a: Hollow cathode lamp emits line spectrum of element to be determined

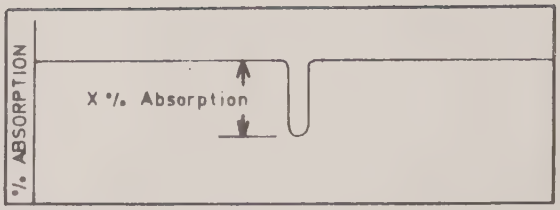

Figure 1b: Sample absorbs energy at the resonance line

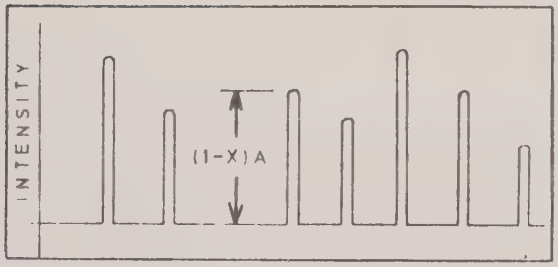

Figure 1c: Resultant spectrum after absorption by sample

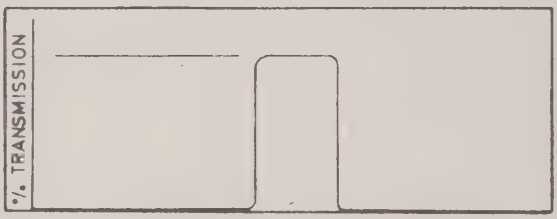

Figure 1d: Monochromator isolates resonance wave-length and rejects all others

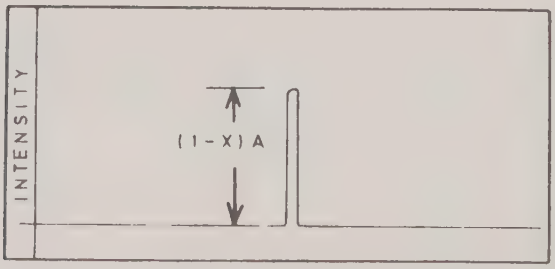

Figure 1e: Photodetector sees only the resonance line diminished by sample absorption

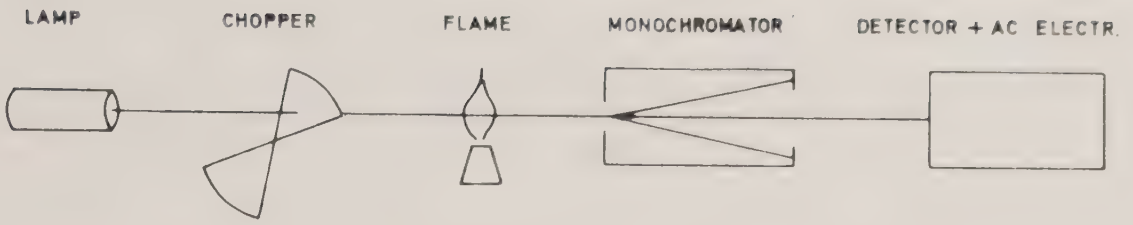

Figure 2 Single beam a.c. system
A chopper distinguishes flame emission from lamp emission. This is the most common system for atomic absorption

(From Kahn, 1968)

the photodetector and electronics. Since the chopped light produces an alternating current in the detector and the electronics are designed to amplify alternating current only, the light from the flame is ignored.

To overcome some of the disadvantages of the single beam system most of the new instruments are designed as double beam systems, shown in Figure 3.

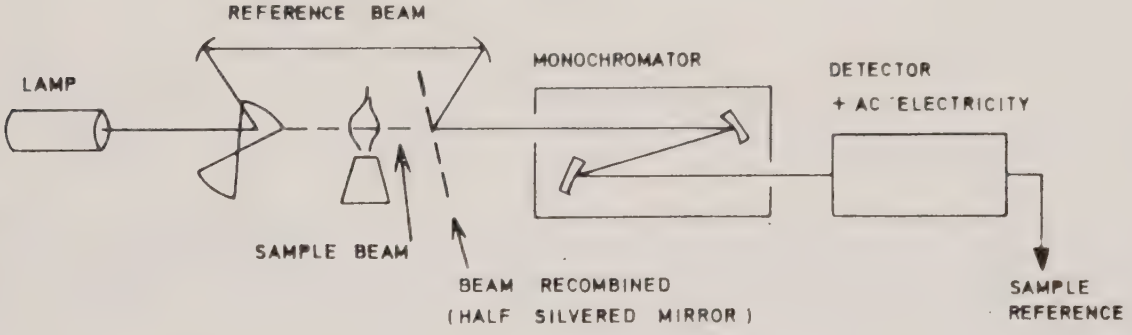

Figure 3 Double beam a.c. system
The effects of variations in lamp emission, detector sensitivity and electronic gain are cancelled out by this system

(From Kahn, 1968)

Here the rotating chopper becomes a rotating sector mirror which passes the beam from the lamp alternately through the flame (sample beam) and past the flame (reference beam). The sample and reference beams are recombined by a half-silvered mirror and then passed together through the rest of the system. The electronics are designed to measure the ratio of the two beams. The readout is the fraction produced by the sample beam as numerator, and the reference beam as denominator. Changes in lamp emission, detector sensitivity and electronic gain appear both in the numerator and in the denominator and are cancelled out. The double beam system thus gives a stable base-line almost immediately, and has the following advantages: lamps can usually be inserted and operated at once; the stability of the base-line makes it possible to observe small deviations from it, and a stable base-line implies good analytical precision. With the double beam, very high values of scale expansion are possible.

9.2.2 Interferences

One of the relatively few interferences found in the atomic absorption spectrophotometry is that of non-atomic absorption. Non-atomic absorption results from the absorption of the hollow cathode lamp radiation by materials in the absorption cell that are extraneous to the element of interest. This type of "false" absorption can lead to erroneous results when trace amounts of an element are determined in complicated sample matrices.

Molecular absorption is caused by the presence of molecular species in the absorption cell which absorb light at the same wave-length as the analyte resonance line. This type of non-atomic absorption varies significantly with wave-length.

Light scattering by particles in the flame results when solutions of high total solids are aspirated into the burner. This type of interference is common in the U.V. portion of the spectrum and becomes more significant at shorter wave-lengths. Light scattering by particles covers a very broad range of wave-lengths.

Both types of non-atomic absorption occur over wide spectral bands (greater than 10 nm) compared with the 0.01 nm absorption band for atomic lines in the flame and are called background absorption, which can be compensated for in the following way.

The absorption measurement obtained with the hollow cathode line source is the sum of an element's atomic absorption and the non-atomic absorption. The absorption measured with the continuum lamp (at the same wave-length) is, to a close approximation, the non-atomic absorption only. The absorption measurements obtained with the continuous source are subtracted from those obtained with the hollow cathode line source to yield the sample's true atomic absorption.

Two types of continuous source are commonly used with atomic absorption spectrophotometers; one is a hydrogen-filled hollow cathode lamp with a nickel cathode and the other is a deuterium arc lamp.

Other interferences found in atomic absorption are chemical and matrix interferences, e.g., caused by other chemical compounds in the sample which influence the degree of reduction to neutral metallic atoms, or the viscosity, surface tension, etc. of the sample solution. These interferences can often be overcome by higher temperature in the flame, by diluting the sample, by adding suitable chemicals or matching the concentration of major constituents in sample and standard solutions.

9.2.3 Detection limits

Detection limits are defined as the concentration in aqueous solution that will produce a signal that is twice the standard deviation of the random fluctuations (noise) of the background (base-line) signal. Some typical detection limits for a double beam instrument are listed below.

9.2.4 Sensitivity

Sensitivity in atomic absorption refers to the concentration in aqueous solution which will generate a signal of 1 percent absorption (0.0044 absorption units). Sensitivities may vary slightly with each instrument as they are a function of the lamp, burner head and nebulizer used. In general one can find the values in the manufacturer's operating instructions.

9.3 Flame emission spectroscopy

There are several basic differences between atomic absorption and flame emission spectroscopy. In atomic absorption, the only function of the flame is to convert the incoming

aerosol into atomic vapour which can then absorb light from the primary light source, the hollow cathode lamp. The flame emission technique requires the flame to do two jobs; convert the aerosol into an atomic vapour and then thermally elevate the atoms to an excited electronic state. When these atoms return to the ground state, they emit light which is detected by the instrument. The intensity of light emitted is related to the concentration of the element of interest in solution.

Table I

Detection limits for the AAS - instrument
(with permission of Varian Techtron Pty. Ltd., Australia)

Ag	0.003	Fe	0.005	Nb	2	Sn	0.004
Al	0.04	Ga	0.1	Nd	2	Sr	0.03
As	0.25	Gd	3	Ni	0.008	Ta	2
Au	0.01	Ge	0.2	Os	0.1	Tb	0.5
B	2	Hf	2	Pb	0.02	Te	0.01
Ba	0.01	Hg	0.2	Pd	0.02	Ti	0.01
Be	0.002	Ho	0.1	Pr	10	Tn	0.02
Bi	0.06	In	0.05	Pt	0.1	Tm	0.02
Ca	0.0005	Ir	0.4	Rb	0.003	U	40
Cd	0.0006	K	0.003	Re	0.9	V	0.1
Co	0.007	La	3	Rh	0.006	W	1
Cr	0.005	Li	0.002	Ru	0.2	Y	0.5
Cs	0.02	Lu	1	Sb	0.07	Yb	0.01
Cu	0.002	Mg	0.0003	Sc	0.05	Zn	0.002
Dy	0.03	Mn	0.003	Se	1	Zr	1
Er	0.05	Mo	0.04	Si	0.3		
Eu	0.02	Na	0.0003	Sm	4		

Most analysts will find atomic absorption easier to use for most of their work. It is easier to optimize the flame for atomic absorption than for emission. Band emission from the flame can cause problems in flame emission which are not present with the atomic absorption technique. In addition, more analytical procedures have been worked out for atomic absorption than for flame emission.

There are, however, certain situations where the use of flame emission may be recommended. It can be used when no lamp is available and the number of samples or available time does not justify obtaining one. Flame emission has better detection limits for some elements. Scanning flame emission can be used for qualitative or semi-quantitative work. A portion of the spectrum is scanned and compared with reference spectra for the presence or absence of certain peaks characteristic of the elements in question.

9.3.1 Selection of experimental parameters

Selection of the proper experimental conditions for an analysis by flame emission involves the balancing of several factors which frequently conflict with each other. One must choose a flame which produces the species being observed, normally atoms, but in the case of sulphur and phosphorus, for example, the S_2 and HPO species. A hot flame is desirable to provide maximum excitation of the spectrum and yet it is desirable to have a minimum of background emission and ionization.

The other factor which must be considered when selecting the analytical wave-length, is the other elements present in the sample. Spectral interferences are somewhat more common in flame emission than in atomic absorption. A good compilation of wave-lengths, such as NBS Monograph 32 or the newly re-issued MIT Wave-length Tables (Harrison, 1969) should be available for reference. The Standard Conditions page for each element suggests several of the more sensitive lines which are suitable for many applications. However, it is impossible to anticipate every possible matrix which may contribute interfering lines.

9.3.2 Interferences

Many of the interferences observed in flame emission are the same as those observed in atomic absorption. Included in this group are matrix, ionization and chemical interferences (see chapter 9.2.2).

An additional problem in flame emission is various interferences which can be classed together under the heading of "spectral" interferences. Included in this group are nearby line, molecular, and self-absorption interferences. Nearby line interferences occur when a line of a second element in the sample is within the spectral band pass of the monochromator. If the amount of this second element is changing, a false change in signal for the element of interest will occur. Interferences of this type are known in atomic absorption but are very few in number and generally confined to the secondary lines. Molecular interferences cause difficulties because a portion of the spectrum is obscured making it difficult to observe the line of interest. It is also possible for the intensity of some band systems to change from sample to sample.

Self-absorption is the cause of non-linearity of calibration curves at higher concentration. The cause is the reabsorption of part of the light emitted in one part of the flame by atoms in another part closer to the monochromator as the light passes through. The greater the atom population, the more likely some of the light will be absorbed.

9.4 References

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10. HEAVY METAL ANALYSIS IN WATER BY COLORIMETRIC METHODS

by

Bernt Röndell

10.1 Introduction

If a beam of white light is allowed to pass through a glass cuvet filled with liquid, the emergent radiation will be less powerful than that entering. The diminution in power may be approximately equal over the whole wave-length range if the liquid is colourless, but it will be to a different extent for different colours. The loss is due in part to scattering by any suspended particles present and in part to reflections at the surface, but is primarily accounted for by the absorption of radiant energy by the liquid. This is the principle for the most widely used technique for the analysis of metals in water and involves the absorption of light by the metals in the form of soluble complexes, chelates or compounds. Although many of these metals at high concentrations do absorb light, it is often necessary to form a stronger light-absorbing species, by using a "colour developing agent" which should, optimally, be specific for the test metal of interest.

The general term for chemical analysis through measurement of absorption of radiation is absorptiometry. Colorimetry should be applied only in relation to the visible spectral region. Spectrophotometry is a division of absorptiometry which refers specifically to the use of the spectrophotometer. This chapter is limited to the discussion of absorption methods in the region of visible radiation, that is, colorimetry, or more correctly, spectrophotometry, concerning the analysis of trace metals in water.

10.2 Molecular absorption spectrophotometry

Although very many organic compounds absorb quite strongly in the visible region, only a limited number of inorganic ions do, and it is therefore necessary to add a molecule or reagent species to the solution of the inorganic ion which will react with, and in the process bring about a marked change in, the spectral absorption characteristics of the reagent.

It is essential that the absorption spectrum of the reagent-ion be well separated at least at one place from the absorption spectrum of the reagent itself. It is common practice to add a fairly large excess of the organic reagent so that virtually all of the ionic species are driven to react with the reagent.

The basic law of absorption spectrophotometry may be expressed as:

$$A = \log(I_0/I) = klc \qquad \text{Lambert-Beer's law}$$

It relates the absorbance (A) to the concentration of the absorbing species (c) linearly through the length of the absorbing layer of solution (l) and the molar absorptivity of the absorbing species (k). I_0 and I are the intensities of the incident and emitted light respectively.

Lambert-Beer's law also applies to a multicomponent system in which there is no interaction between the various species. The total absorbance may be given as:

$$A_{total} = k_1lc_1 + k_2lc_2 + k_3lc_3 \ldots$$

where (k) denotes the molar absorptivity of each of the absorbing species, and (c) represents its molar concentration. This equation is the basis of all work done on mixtures of absorbing substances.

In many instances a wide choice is open to the analyst as to which type of apparatus to employ. Thus copper can be determined, with varying degrees of precision, by the ammonia-colour method with Nessler tubes, a Duboscq comparator, a filter photometer, or the most elaborate spectrophotometer. A generalized diagram of a double beam spectrophotometer, a rather elaborate apparatus, is shown in Figure 1.

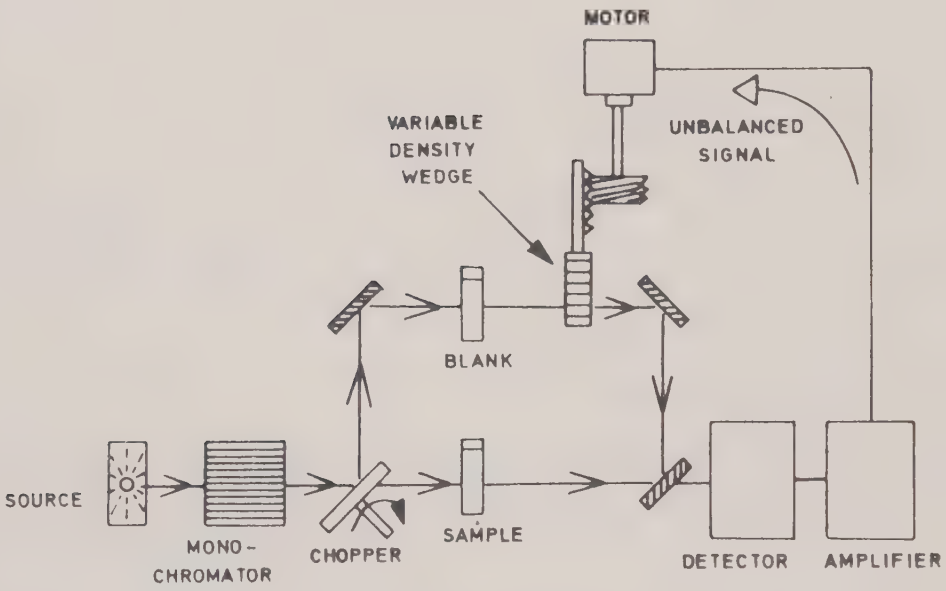

Figure 1. Double beam-null balance spectrophotometer (from: Mancy, 1971)

In this type of instrument arrangement, one beam provides a reference signal, the other the measured signal. The blank is placed in the first beam, the sample in the second. By referring intermittently or continuously to the reference beam when making measurements, errors that arise from fluctuations and drift in the applied voltage, source intensity, detector response, amplifier gain, and other irregularities, as well as errors that the blank is particularly designed to compensate for, are largely eliminated. A detailed discussion of instrument arrangements, spectrophotometer components, including various commercial instruments available, is given by Willard et al. (1965) and in other standard books.

10.3 Separation and formation of absorbing compound

There are generally several methods that can be used for the spectrophotometric determination of a given substance. Careful selection of a method plays an important role in the successful analysis of a sample. The absorbing substance to be determined should be selectively separated, if necessary, for the absorbance measurement. Some of the more common organic reagents used for separation by extraction are chelate compounds, e.g., dithizone (diphenylthiocarbazone), oxines, cupferron and diethyldithiocarbamate. Examples of the applications of this technique for analysis of metals in natural and waste waters are given

in Table I. The reagents should be selective in their reactions and should not form interfering absorbing species with foreign substances likely to be present. The reagent must be sensitive enough to react in the desired concentration range of the absorber in the unknown samples.

Table I

Examples of molecular absorption spectrophotometry for metals in natural and waste waters (from: Mancy, 1971)

Metal	Complexing Agent	Solvent Extraction	Colour of Complex	pH Range	Suitable Wave-length nm	Useful Range mg/l
Cobalt	diethyldithio-carbamate	ethyl acetate	blue	acidic pH 3.0	367	-
Cadmium	dithizone	carbon tetrachloride	red	alkaline pH 10-12	518	0.1-5
Chromium	1.5 diphenyl carbohydrazide	butanol	violet	acidic pH 2-3	540	0.05-0.5
Copper	dithizone	carbon tetrachloride	violet	acidic pH 0.5	510	0.04-14
Copper	diethyldithio-carbamate	carbon tetrachloride	yellow-brown	alkaline pH 9.0	436	0.1-0.8
Iron	o-phenanthroline	-	orange-red	pH 2-9	490	0.01-1
Iron	thioglycollic acid	-	purple	pH 8-12	540	0.04-1
Iron	tripyridyl	-	red-purple	pH 9-10	560	0.01-2
Lead	dithizone	chloroform	red	pH 7-10	520	-
Mercury	dithizone	carbon tetrachloride	yellow-orange	pH 0-1	500	-
Nickel	dimethyl-glyoxime	-	reddish-brown	neutral	465	-
Zinc	dithizone	chloroform	purple-red	pH 4-5.5	530	0.1-1.0
Zinc	zincon	-	blue	pH 9.0	620	0.1-2.4

The common and important variables for colour development are:

(a) pH. Since pH is so important in complex formation, proper adjustment of pH often favours the formation of the complex desired instead of interfering ions. Thus it is necessary to control the pH critically by use of suitable buffers.

(b) Concentration of reagents. An optimum concentration of reagents should be determined; insufficient or excess reagent can cause deviation from Lambert-Beer's law. The amount of reagent required is dictated by the composition of the absorbing complex formed. Too much reagent may cause a high blank or the formation of complexes with interfering substances.

(c) Time. Full colour development is sometimes not immediate but requires several minutes or a few hours of standing time.

(d) Order of mixing reagents. In certain cases, the order in which reagents are added is important. Otherwise, no full colour development will be possible or interfering reactions may occur.

(e) Temperature. Certain reactions require elevated temperature to hasten them. The optimum temperature should be determined.

(f) Stability. If the complex formed is not very stable, the absorbance measurement should be made as soon as possible. If the coloured complex is light sensitive, precautions to prevent decomposition should be taken; perhaps addition of another reagent may help.

(g) Masking. Few reactions are really specific. However, compounds such as EDTA may often be added to the sample to form a stable complex with interfering ions, thus preventing their reaction with the colour reagent.

(h) Organic solvent. Many reagents or complexes of organic nature are sparingly soluble in water. It is sometimes necessary to add a water-miscible organic solvent such as ethanol to help colour development if solvent extraction is not desired. However, solvent extraction often permits the removal of interfering ions.

(i) Salt concentration. The presence of high electrolyte concentrations often influences the absorption spectrum of a compound, forming ion association complexes that cause a shift in the absorption maximum.

(j) Preparation of Lambert-Beer's law plot. The absorbances of several coloured solutions of known but varying concentrations are measured in preparing an analytical curve. Conditions are adjusted for optimum colour development and the absorbance of each solution is measured at the selected wave-length. Under these conditions a system that conforms to Lambert-Beer's law gives a straight line when a graph of absorbance versus concentration is plotted. The slope is the product of the molar absorptivity, k, and the path length, l.

(k) Precision, accuracy. The precision and accuracy of a photometric procedure vary considerably with the type of instrument used, the chemistry of the system being studied, and the care taken. When a new photometric procedure is developed, its precision should be tested for the concentration range of the substance to be determined. The accuracy of a procedure may be determined by using either synthetic mixtures or standard samples.

10.4 Applications for analyses of metals

Being present in very low concentrations, the analysis of metal elements always presents problems. Contamination, in particular, can very easily occur. Analyses can be made on samples which have been filtered, filtered and digested, not filtered, not filtered and digested. In relation to living organisms, the first two of these are likely to be the most informative.

However, it must be remembered that colorimetrical analyses are performed at specified pH intervals which must be carefully controlled. Digested samples most often contain great amounts of acid, thus requiring considerable amounts of buffering substances to be added for the pH control. The buffering substances always contain small amounts of heavy metals even if very pure chemicals are used. This extra addition of metals to the samples will cause problems if the original metal contents of the samples are low. Thus, for the analyses of digested samples, the atomic absorption or flame emission techniques are preferred because they are directly applicable to strongly acidic samples.

10.4.1 Aluminium

Probably the most widespread analysis for aluminium utilizes its ability to unite with an ammonium salt of aurin tricarboxylic acid ("aluminon") and form a red to pink complex which exhibits maximum absorption near 525 nm. Many ions interfere in this reaction, such as fluoride polyphosphate, sulphite, chlorine, calcium and iron (American Public Health Association, 1965; Packham, 1958). Another method uses Eriochrome cyanine R, which produces a red to pink complex which exhibits maximum absorption near 535 nm at a pH of 6.0. Negative errors are caused by both fluoride and polyphosphates (American Public Health Association, 1971). Ferron (8-hydroxy-7-iodo-5-quinoline sulphonic acid) also reacts with aluminium to

give a soluble, intensely coloured complex. Iron interferes seriously, but this effect can be minimized by adding an iron-complexing agent, such as ortophenantroline. Manganese, lead, cobalt and fluoride also interfere (Brown et al., 1970). The method outlined in chapter 22.1 utilizes Pyrocatechol violet as the colour-producing agent (Wilson and Dougan, 1972).

10.4.2 Cadmium

Cadmium ions, like those of many other metals, react under suitable conditions with diphenylthiocarbazone (dithizone) to form a red compound which can be extracted into chloroform and used for the colorimetric determination of cadmium in natural waters. Concentrations of other metal ions normally found in water do not interfere. In polluted waters, silver, mercury, copper, nickel, cobalt and zinc interfere and have to be removed by preliminary extractions. The final extraction is carried out from a strongly basic medium. If the zinc-cadmium ratio is greater than 500:1, it is difficult to extract the last traces of cadmium and low results are obtained (American Public Health Association, 1971; Fisher and Leopoldo, 1937; Great Britain, Department of the Environment, 1972).

10.4.3 Chromium

In acid solution, diphenylcarbazide and chromium (VI) form a soluble red-violet product that absorbs light at 540 nm. This reaction can be used to determine soluble chromium (VI) in natural waters. For all practical purposes the reaction is specific for chromium. Iron, mercury and molybdenum interfere, so does vanadium which should not be present in concentrations exceeding 4 mg/l (American Public Health Association, 1971; Brown et al., 1970). However, in natural waters and moderately polluted ones, these metals are rarely present in concentrations high enough to interfere.

All ionic forms of chromium can be determined by oxidizing chromium (III) to chromium (VI) with potassium permanganate prior to the addition of diphenylcarbazide. The excess oxidant is destroyed with sodium azide. An alkaline hypobromite solution can also be used as oxidizing agent; organic matter, however, should be absent (American Public Health Association, 1971).

10.4.4 Copper

Cupric ions react with the reagent bis (2-hydroxyethyl) dithiocarbamate (cuprethol) to form a yellow-coloured chelate. The soluble coloured compound is formed quantitatively and may be used to determine copper in relatively unpolluted waters. Bismuth, cobalt, mercury, nickel and silver must be absent (American Public Health Association, 1971).

In polluted waters the bathocuproine or the neocuproine method may be used. The first utilizes the ability of the cuprous ion to form a water-soluble, orange-coloured chelate with bathocuproine disulphonate. Hydroxylamine hydrochloride serves as a reducing agent and the system is buffered to a pH of 4.3 (American Public Health Association, 1971). The second method utilizes 2,9-dimethyl-1,10-phenantroline (neocuproine) as the complexing agent. It reacts with the cuprous ion in neutral or slightly acidic medium and the complex formed can be extracted by a number of organic liquids, including a chloroform-methanol mixture. The reaction is virtually specific for copper (American Public Health Association, 1971).

For sea water, Strickland and Parsons (1968) recommend diethyldithiocarbamate as a reagent for copper. The yellow complex is extracted into carbon tetrachloride. The method outlined in chapter 22.4 can also be used for sea water (Nilsson, 1950; Tuck and Osborn, 1960). Another reagent that may be used for copper determination in sea water is cuprizon (bis-cyclohexanone-oxalyl-dihydrazone). It gives a very sensitive blue colour reaction with copper in weakly alkaline solution and no extraction is necessary (Wetlesen and Gran, 1952).

12.4.4 Iron

One of the most used methods for determining iron in natural and treated water is the phenanthroline method. Iron is reduced to the ferrous state by boiling with acid and hydroxylamine, and treating with 1,10-phenanthroline to form an orange-red complex. Strong oxidizing agents, cyanide, nitrite, phosphates and some metal ions interfere. However, an extraction step can be included and the effect of interfering species minimized (American Public Health Association, 1971). Alternatively, in the presence of interfering substances, the "ferrozine" method may be used. 2,2'-bipyridine reacts with ferrous iron to form a red complex (Brown et al., 1970), and 2,2',2"-tripyridine reacts to form a reddish-purple complex (American Public Health Association, 1971). These methods are subject to the same type of interferences as the phenanthroline method, although the amounts of interfering ions can be higher.

For low concentrations of iron, the bathophenanthroline reagents have a sensitivity about twice that of orthophenanthroline. The colour complex that ferrous iron forms with bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) has been used to determine soluble iron in sea water (Strickland and Parsons, 1968; Gosh et al., 1967). The method outlined in chapter 12.2.3 has also been used in sea water (Collins and Diehl, 1960).

12.4.6 Lead

Lead is another metal that reacts with dithizone. It forms a red complex, lead dithizonate, in carbon tetrachloride solution. Measurement of the amount of red colour formed yields an estimation of the lead present. The procedure is extremely sensitive. Interfering metals are removed by double extraction at controlled pH. Organic material must be removed prior to the analysis (American Public Health Association, 1971).

12.4.7 Manganese

Manganese is readily determined in small concentrations by oxidizing lower valent Mn (valence states II, III and IV) in acidic solution to permanganate. The absorbance is then measured colorimetrically. Either periodate or persulphate can be employed as the oxidizing agent. The latter is usually preferred for two reasons: the use of mercuric ion can control the interference from a limited chloride ion concentration, and the method is also more rapid in the presence of low manganese concentrations (American Public Health Association, 1971; Kowalski, 1967; Great Britain, Department of the Environment, 1972).

Another procedure for total manganese is the catalytic method which makes use of the formation of the dye malachite green. It is one of the most sensitive colorimetric methods known for dissolved manganese. The method employs oxidation of the leuco-base of malachite green by periodate at pH 8 with Mn acting as a catalyst. The method has been used in oceanographic work (Strickland and Parsons, 1968; Manheim and Gordon, 1969). The method outlined in chapter 12.3 is based on the reaction of manganese with formaldoxime in basic medium (Delphine and Lee, 1960; Morgan and Stumm, 1965).

12.4.8 Mercury

Mercury also reacts with dithizone; however, the procedure is less satisfactory than for many of the heavy metals, due in part to the relative lack of sensitivity. The mercury is extracted by extraction with excess of a solution of dithizone in carbon tetrachloride. The mercury is removed from this extract and returned to the aqueous phase by oxidation with sodium nitrite. After the addition of EDTA, which hinders the reaction of copper with dithizone, the mercury is finally extracted again with dithizone in carbon tetrachloride and determined colorimetrically. Organic material must be removed prior to the analysis, and that must be done carefully to avoid the possible loss of mercury due to the volatility of the metal and its covalent compounds. (Great Britain, Department of the Environment, 1972.)

10.4.9 Nickel

Nickel reacts with dimethylglyoxime in the presence of an alkaline oxidizing agent and can thus be determined colorimetrically. In the presence of citrate, iron, manganese and copper cause little interference at concentrations up to two or three times the nickel concentration. When greater concentrations are present, the interference can be eliminated by extracting the nickel dimethylglyoxime complex with chloroform.

Another method for determining nickel uses heptoxime instead of dimethylglyoxime to develop the colour. In this method also copper and iron interfere and are removed by extraction with chloroform (American Public Health Association, 1971).

10.4.10 Tin

Tin forms a red complex with catechol violet in slightly acid solution. Tin as stannic iodide is selectively extracted by toluene and is returned to aqueous solution by extraction with dilute sodium hydroxide. After pH-adjustment the dye is added and the intensity of the colour measured. The method is not subject to interferences by other metals likely to occur in effluents and water. (Great Britain, Department of the Environment, 1972.)

10.4.11 Zinc

Zinc is the fourth metal discussed here that can be determined using dithizone. Specificity in the separation is achieved by extracting from a nearly neutral solution containing bis (2-hydroxyethyl) dithiocarbamyl ion and cyanide ion, which prevents moderate concentrations of cadmium, copper, lead and nickel from reacting with dithizone. Either the red colour of the zinc dithizonate or the green colour of the unreacted dithizone can be measured (American Public Health Association, 1971).

Also zincon (2-carboxy-2′-hydroxy-5′-sulphophormazyl benzene) can be used for the determination of zinc. A blue-coloured complex is formed in a solution buffered to pH 9.0. Heavy metals, including zinc, are first complexed by adding cyanide to the sample. Chloral hydrate is then added specifically to free the zinc from its cyanide complex. The final solutions are unstable and the procedure is designed to minimize the effects of colour fading (American Public Health Association, 1971; Platte and Marcy, 1959).

10.5 Other methods for heavy metals

Many other methods exist and are used daily for the metals mentioned above. Colorimetric methods also exist for almost every other metal. They have the main advantage that they usually require relatively inexpensive or virtually no instrumentation. In some cases they are as accurate, precise and sensitive as, for example, atomic absorption spectrophotometry. New methods of colorimetric analysis are always under development. Reviews in each branch of analytical chemistry are published regularly in the periodical, Analytical Chemistry (Fishman and Erdmann, 1971).

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11. INTRODUCTION TO CHROMATOGRAPHIC METHODS WITH SPECIAL REFERENCE TO ANALYSIS OF PESTICIDES AND OIL POLLUTANTS IN THE ENVIRONMENT

by

Stig R. Carlberg
Sören Jensen
Lars Renberg
Reggie Vaz

11.1 Introduction

When substances in a mixture are to be separated, partition of the compounds between two immiscible liquids may often be used. However, when the chemical properties of the compounds differ very slightly, such simple methods are not sufficiently efficient. Chromatographic methods are then very useful tools that can easily be modified for individual separation problems. The word chromatography is derived from its first applications which were to separate coloured compounds.

Three main purposes exist for these techniques: firstly, to isolate compounds which are to be studied. This is usually called a clean-up procedure. Secondly, to separate compounds, which are all to be studied, from one another; and thirdly, to characterize compounds, e.g., retention times in gas chromatography, R_f-values in thin layer chromatography, etc. This is helpful in the identification of a substance.

11.2 Definition of chromatography

Chromatography is the process by which a mixture is separated by adsorption or solution partition between two immiscible phases, one of which is moving and the other stationary.

11.3 The principles of the different chromatographic separation techniques

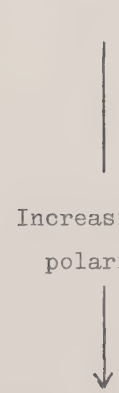
For the moving or mobile phase, only gas and liquid may be considered, while the stationary phase may be either liquid or solid. Therefore, there are four possible chromatographic methods:

(a)	gas - solid	gas chromatography
(b)	gas - liquid	gas chromatography
(c)	liquid - liquid	partition chromatography
(d)	liquid - solid	adsorption chromatography, ion-exchange chromatography and gel permeation chromatography

In this paper, (b) and (d) - in the form of thin layer chromatography - will be discussed in particular, as these two techniques are used for the analyses of pesticides and oil pollutants in this Manual, Sections 17.4 and 17.9.

11.3.1 Adsorption chromatography

This is the most common method used in pesticide analysis and is technically carried out as column chromatography or thin layer chromatography. The substances are separated in order of their polarity. The adsorbent is usually silica gel, aluminium oxide, celite or Florisil. A list of solvents, commonly used in adsorption chromatography and arranged according to their different degree of polarity (the eluotropic series) is given below (the solvents may be used either one by one or in various mixtures as required):



Hexane
Heptane
Carbon tetrachloride
Benzene
Chloroform
Methylene chloride
Diethyl ether
Acetone
Ethanol
Methanol
Water

As a rule, non-polar solvents are used to separate lipophilic substances from each other.

11.3.2 Partition chromatography

Technically, partition chromatography is carried out in the same way as adsorption chromatography but the solid phase is here coated with a liquid. In pesticide analysis this liquid phase is usually a hydrocarbon such as paraffin or a silicon polymer. The solid material thus serves only as a carrier for the liquid stationary phase and the substances are separated according to their different partition coefficients between the moving and the stationary liquid phases.

11.3.3 Gel permeation chromatography

The substances are separated according to molecular size. The smaller molecules are more retarded in the network of the gel than the large molecules. This separation method is commonly carried out using column chromatography. The advantage is that several separations can be carried out using the same column.

11.3.4 Ion-exchange chromatography

This method mainly separates ionized substances according to the differences in strength of the ion bonding. This separation technique is not commonly used as most pesticides and mineral oils are not easily ionized.

11.4 The chromatographic separation techniques

Different techniques for carrying out the separations mentioned above are:

Column chromatography (CC)
Thin layer chromatography (TLC)
Paper chromatography (PC)
Gas chromatography (GC)

11.4.1 Column chromatography (CC)

A glass tube (column) is mounted vertically and filled with the stationary phase, either dry or as a suspension. The substances, normally dissolved in a solvent with a low elution ability, are placed on the top of the column. Finally a solvent reservoir is connected to the top. With a proper choice of conditions the substances will separate during the passage through the column and can be collected in different fractions.

11.4.2 Thin layer chromatography (TLC)

In this technique the stationary phase is a solid (e.g. silica gel) applied as a thin layer - 0.25 mm - on a glass plate. The sample is applied as drops of a more or less concentrated solution. This addition is made as a spot, a few centimetres from one edge of the plate, together with the sample, but as a separate spot. A standard is also applied.

When the spots have dried the plate is placed in a development chamber (see Figure 1). This chamber is a glass container with a tightly fitting lid. Three sides of the chamber are lined with a sheet of filter paper. Fifty ml of a suitable solvent is added to the chamber. The filter paper becomes saturated with the solvent thus ensuring a uniform atmosphere in the entire volume of the chamber.

The glass plate is introduced into the chamber and placed so that the edge with the samples is immersed into the solvent. The surface of the solvent should not reach up to the spots.

The solvent will now ascend along the stationary phase, carrying the samples. However, the components of the samples will not move with the same speed as that of the solvent. The speed is determined by the strength with which the components are adsorbed to the stationary phase.

The distance travelled by each substance is a characteristic and reproduceable quantity for the substance if the governing factors are carefully controlled. Thus the measured distance serves as a means of identification (see definition of R_f values below). But this technique is not so specific that one single run is proof of the presence of each substance. When the solvent front has moved a desired distance along the plate, the plate is removed from the development tank and the absorbed solvent is allowed to evaporate. The result of the separation is rarely visible directly, so some means for visualization must be used.

There are two principal ways of detecting substances on the thin layer plate:

(i) Visualization by UV-light. If the substances absorb long wave UV-light (about 360 nm), e.g., optical brighteners, or short wave UV-light (about 254 nm), such as aromatic substances in general, they can easily be visualized by looking at the plates in suitable UV-light (observe that <u>direct</u> radiation is very harmful to the eyes). However, to visualize compounds in short wave UV light, it is necessary to have a fluorescence indicator incorporated in the thin layer. The substances will then appear as dark blue spots on a light green background;

(ii) Visualization by spraying a reagent on the plate. There are two types of spraying reagents; (a) specific reagents for a special compound or a class of compounds, and (b) general reagents such as 50% sulphuric acid in methanol which, after heating, will carbonize the compounds. The substances appear as grey or black spots.

A variant of this second method is to place the plate in a second development tank which, instead of solvent, contains some iodine crystals. The iodine vapour will adhere to the substances contained in the spots, which appear as yellow to dark brown areas against a light yellow to light brown background. This technique is very useful for samples of mineral oils.

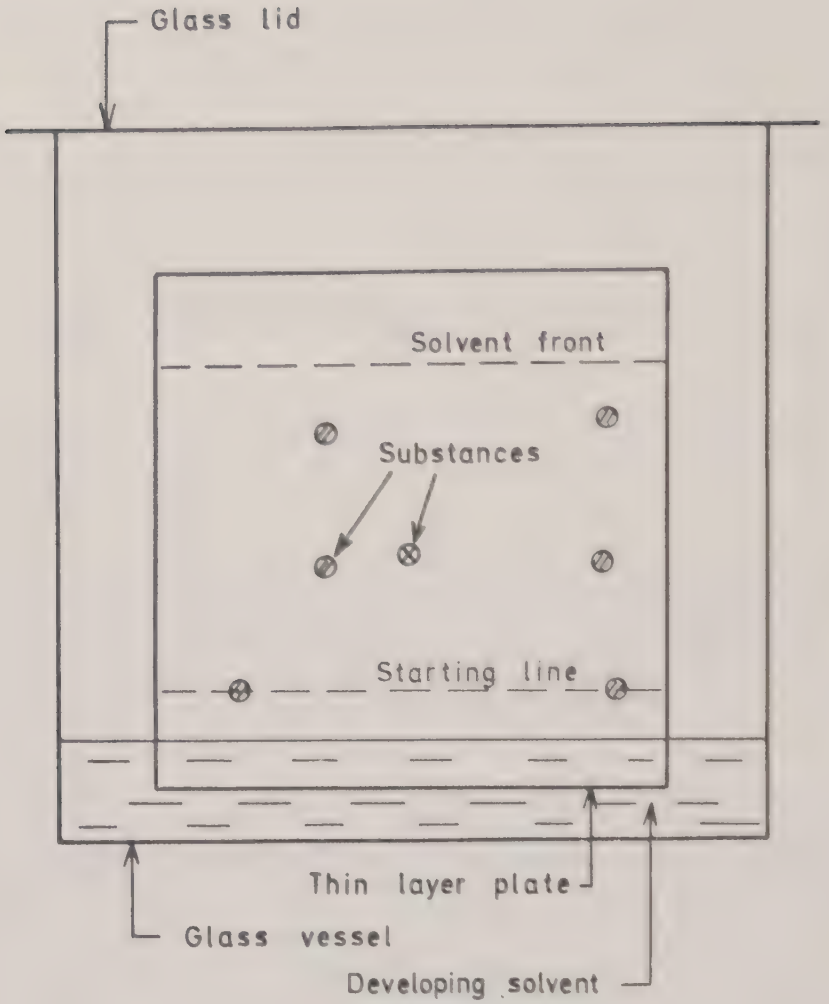


Figure 1 Development tank containing a thin layer plate

The R_f value which characterizes the components is defined as:

$$R_f = \frac{\text{distance from starting point to the component}}{\text{distance from starting point to the solvent front}}$$

A semi-quantitative estimation of the amount of substance present in each spot is possible by comparing the intensities of the sample spots with those spots which contain known amounts of standard.

11.4.3 Paper chromatography (PC)

Paper chromatography is performed in a similar manner to TLC. Instead of a thin layer plate, a porous paper is used which will give partition chromatographic separation with cellulose-water as the stationary phase. The technique is mostly applied to hydrophilic compounds and it is not very much used in analyses of pesticides or mineral oils.

11.4.4 Gas chromatography (GC)

In the gas chromatograph the sample is volatilized at an elevated temperature. A carrier gas (usually nitrogen or helium) transports the components into the column which is contained in an oven. In gas-liquid chromatography (GLC) the column contains a support material which is coated with a liquid stationary phase. This phase is so chosen that the components of the sample are soluble in the phase as well as in the carrier. Every component has a characteristic solubility in the liquid and in the gas. Thus a partition of each component takes place between the two media. As the carrier gas passes through the column during the entire analysis, the components are transported and eluted from the column by the gas. The components which are most volatile in the gas will be eluted first and those which are more soluble in the liquid will come later.

When leaving the column, the components enter the detector (electron capture or flame ionization detector). The detector gives an electrical response for the components. This electrical output is amplified and fed to a strip chart recorder which delivers a continuous plot of detector response versus time. When all governing conditions are kept constant, the time from sample injection to the appearance of a component peak - the retention time - is constant too. Thus, the retention time is used as a means of identification of the components. The area under the peak is proportional to the amount of the eluted compound. By comparing the retention time and peak area of a compound in the sample with that of an injected standard of known concentration the level present in the sample can be estimated. Often peak heights instead of peak areas are used.

The principal layout of a gas chromatograph is shown in Figure 2. A short description of the different parts of the instrument is given below.

The sample is injected into the injection block with a syringe. Here the sample meets the carrier gas stream at an elevated temperature. The sample is volatilized and all components are swept into the column. If the sample contained compounds of high boiling points the injection block must be maintained at a higher temperature than that of the oven. Otherwise compounds will condense and maybe not reach the column. For this purpose the injection blocks are equipped with separate heaters.

The column contains a support material which is impregnated with the liquid stationary phase. The compounds of the sample dissolve in this phase, but as they are soluble in the carrier gas too, the compounds will be partitioned between the two media. By using different stationary phases in various amounts, the separating efficiency of the column (and also the retention times of the compounds) can be greatly modified in order to reach optimal conditions for the analytical separations. The carrier gas velocity, the length of the column and its working temperature are other regulating factors.

The working temperature of the column is provided by the analytical oven which must give an even temperature throughout its entire volume. To ensure this a fan continuously operates in the oven. As the components are eluted from the column, they reach the detector. For the detection of most organic compounds a flame ionization detector is used. This detector is used to measure an ionization current in a hydrogen flame. When an eluted substance is burnt in the flame, an increase in ionization current occurs: the signal is fed to an amplifier and then to a recorder. This is by far the most widely used detector, responding with high sensitivity to almost all organic compounds. The sensitivity of the flame ionization detector (FID) enables 10^{-9} g of many components to be detected, and the linear range of approximately 10^{7} is much wider than that obtained with other detectors.

For the detection of chlorine-containing organic substances (e.g., PCB, DDT and their derivatives) the highly sensitive electron capture detector (ECD) is preferred. This detector consists principally of a radioactive foil that emits beta-particles. These collide with the molecules of the carrier gas and release electrons (=current). A voltage is applied to the detector and the current is reproduced by a 1 mV recorder as a constant baseline. When a chlorinated substance enters the detector, the molecule attracts electrons.

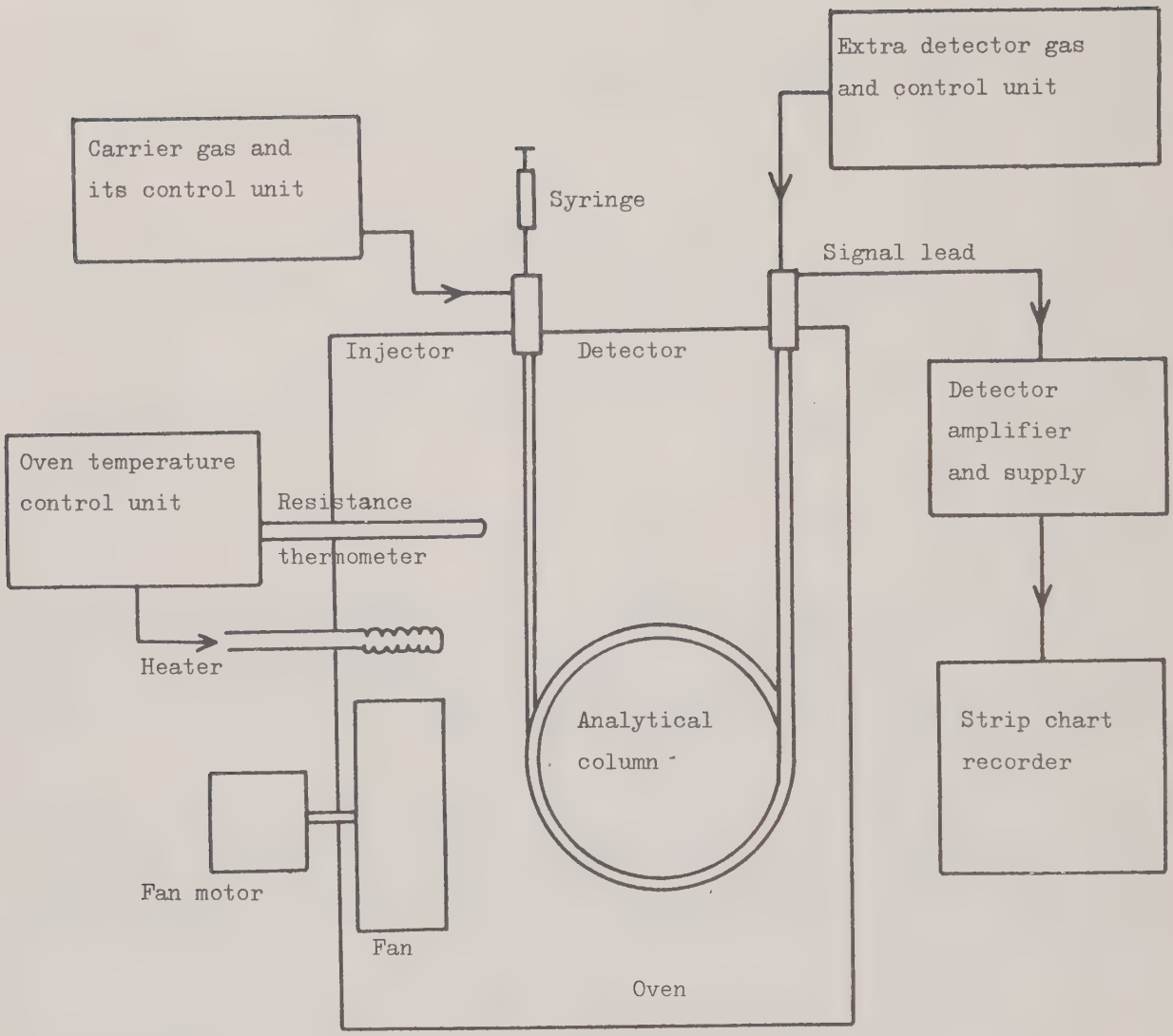


Figure 2 Principal layout of a gas chromatograph, showing the important components (from Andrews, 1970)

As a result the current drops, which is indicated as a peak by the recorder. The ECD is at present capable of detecting ppb levels (ppb = parts per billion, e.g., 10^{-9} g substance/g sample) or picogram amounts (picogram, pg, = 10^{-12} g).

In addition to these major parts, some auxiliary equipment is required. The carrier gas is fed from its supply via a gas regulator and a measuring device. The flow of the carrier gas must be carefully controlled as it is one of the governing factors of the retention time. If the compounds in the sample do not differ very much in their boiling temperatures, it is usually possible to perform the analysis under isothermal conditions, which means that the working temperature of the column is not changed during the analysis. This is normally the case with the pesticide analyses, which are performed at a column temperature of about 200°C, with the injection block and detector temperatures held at 20-50°C higher.

If the sample contains components covering a great range of boiling points - and this is the case with samples containing mineral oils - it is not possible to carry out the analysis at one and the same column temperature. If the temperature chosen is too low, the high boiling components will never be eluted, or eluted after an intolerably long time. On the other hand, if the temperature chosen is too high, the low boiling components will be eluted so quickly that no separation of them takes place. To solve this problem a temperature programmer is used. This enables the analyst to start the analysis at one low temperature, holding this constant if necessary for a certain time, then increasing the temperature at a carefully controlled rate up to a final temperature, which in turn may be kept constant for another specified time. After this the oven is cooled down to the initial temperature before the next sample is analysed.

The eluted components - especially those with high boiling points - must be prevented from condensing in the detector, because this inevitably leads to contamination and loss of sensitivity. In order to prevent this condensation the detectors are contained in separately controlled ovens of their own.

The FID uses a hydrogen flame for burning of the eluted substances. For this flame hydrogen and compressed air must be supplied. Also these gases are led from their supply tubes via gas flow controls and measuring devices.

As was pointed out earlier, the retention time for a compound is a characteristic, if temperature, carrier gas flow and other governing factors are kept constant. Unfortunately several compounds can have the same retention time. For definite identification of a substance other instruments such as the mass spectrometer are necessary (Figure 3)

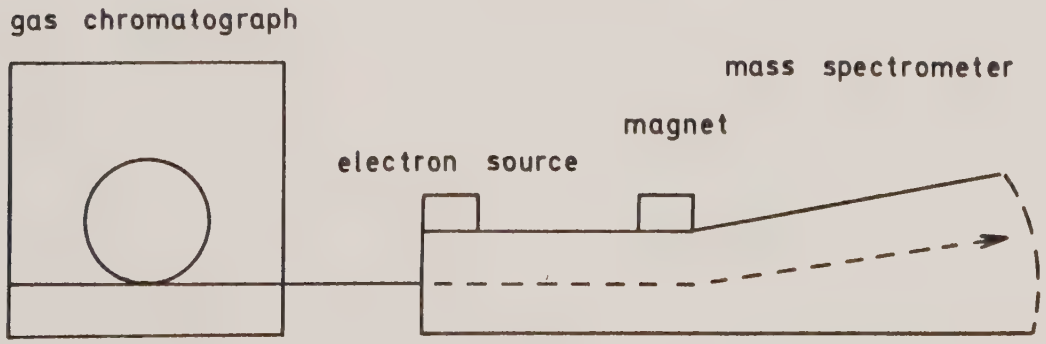


Figure 3 Simplified diagram of a mass spectrometer
(from Olsson and Jensen, 1971)

The molecule, after leaving the gas chromatograph, enters the mass spectrometer. The molecule is bombarded with electrons from an electron source, which will result in loss of an electron or a fragmentation of the molecule. The positive charged fragments, including the molecule ion, are accelerated in a vacuum. The magnet deflects the ionized fragments, the degree of deflection depending on the molecular weight (or rather the analogous term "fragment weight"). Thus the molecular weight and "fragment weights" can be found and the chemical structure of the compound determined.

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12. ANALYSIS OF CHLORINATED HYDROCARBONS IN THE ENVIRONMENT

by

Sören Jensen

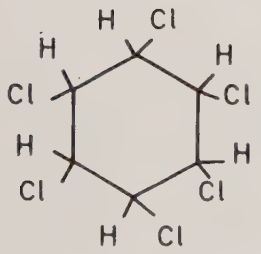
12.1 Evaluation of the most important chlorinated hydrocarbons in the environment

The chlorinated compounds most often found in residue analysis of organisms belong to the following classes.

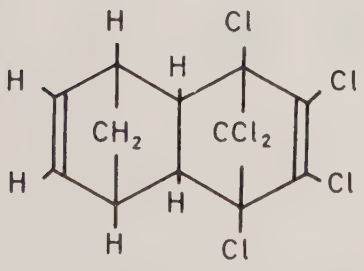
12.1.1 Alicyclic compounds

This group covers the hexachloro cyclo-hexane isomers (BHC). The γ isomer is called lindane. The half-life in organisms is rather short, and thus they are only found in biological material after heavy use in small areas. Organisms from the deep sea only very rarely contain lindane.

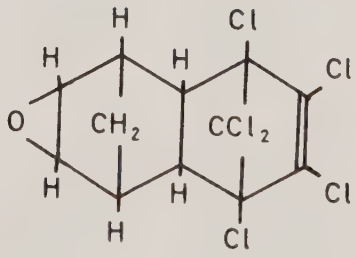
The chlorinated dienes such as aldrin and dieldrin (and the isomeric endrin) also belong to the alicyclic class (Figure 1). Both are used as pesticides in agriculture and the latter also in large amounts to protect wool against moths. In residue analysis dieldrin is found to be present more often than aldrin. The difference between them is that a double bond in aldrin is exchanged for an epoxide ring. This change is also the first step in the degradation of aldrin in nature. The epoxide ring is further oxidized to dihydroxy compounds.



Hexachlorocyklohexane
(lindane - γ-isomer)



Aldrin



Dieldrin

Figure 1. Alicyclic compounds

12.1.2 Chlorinated diphenyl ethanes

This group includes the DDT-family. DDT is dichloro-diphenyl trichloroethane. In almost every biological sample analysed anywhere in the world, at least trace amounts of DDT and its metabolites DDD and DDE (dichloro-diphenyl dichloroethane and dichloro-diphenyl dichloroethene, respectively) are found (Figure 2). In biological samples, DDT itself is only present in larger amounts than its metabolites in cases where the organisms are collected close to the place where DDT is used. In all other samples DDE is the major component. DDE seems often to be formed as the end product in higher organisms (no other significant metabolite is found) while DDD, on the other hand, is formed in micro-organisms which seem unable to form DDE. DDD is further metabolized to DDA (dichloro-diphenyl acetic acid) via the corresponding alcohol and aldehyde. This metabolite is very seldom included in a residue analysis. Probably it does not accumulate to the same degree as DDT, DDD and DDE because of a greater hydrophility. DDT, as it is used in agriculture, consists of the two isomers, para para (p.p'.) and orto para (o.p.). The composition is often 75% of the former and 25% of the latter. In highly polluted areas o.p.-DDT is often found and sometimes also its metabolites o.p.-DDD and o.p.-DDD. But these compounds seem to be less stable than the p.p'.-isomers. It has been suggested that o.p.-DDT can be converted to the p.p'.-isomer in the environment.

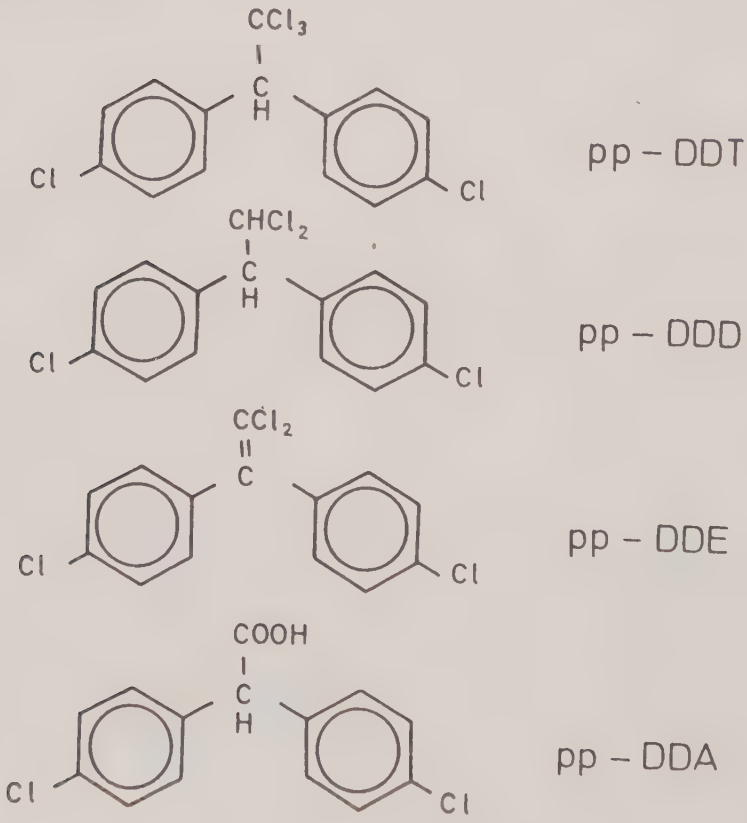


Figure 2. Chlorinated diphenyl ethanes

12.1.3. Polychlorinated biphenyls (PCB)

These compounds, in contrast to all the above-mentioned, have no aliphatic part in the molecule (are purely aromatic) and thus seem to be extremely difficult to metabolize. They consist of a large number of homologues and isomers. 210 different chlorinated biphenyls are possible, but PCB found in nature most often contains about 50% chlorine per molecule, that is, from 4 to 8 chlorine atoms per molecule (Figure 3). Assuming an even distribution of chlorine on both the aromatic nuclei, 104 different PCBs are theoretically possible and of these 14 are often indicated in residue analysis. PCB has a purely industrial history and possibly finds its way to the environment through waste water discharges or from chimneys through which it passes because of its non-inflammability.

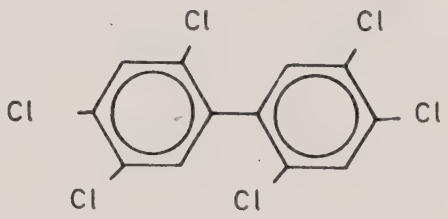
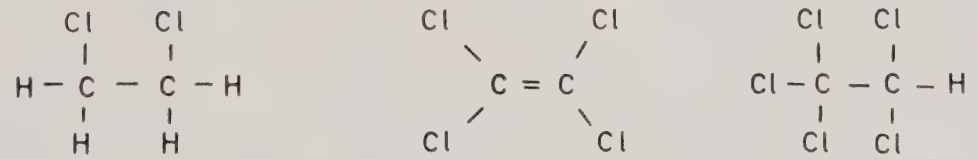


Figure 3. A typical component in PCB

12.1.4 Aliphatic chlorinated hydrocarbons

The last group of chlorinated hydrocarbons, found in residue analysis very recently, is the aliphatic chlorinated hydrocarbons. These substances are used on a very large scale as solvents. The most important are chloroform, carbon tetrachloride and dichloroethane. Many others are formed, for instance in the production of vinyl chloride used in PVC manufacture (Figure 4). These by-products in the order of 100 000 tons, are discharged into the environment (often into the deep sea) where they can be accumulated in living organisms. The contamination level in plankton can be several hundred times higher than in the water. The exact composition is still under investigation, but it seems clear that the carbon chain contains from 2 to 4 carbon atoms and the chlorine content is often from 2 to 5 per molecule. The biological half-life of this group of substances is low in relation to the above-mentioned classes, and the half-life in fish transferred to pure water is for many of them counted in days instead of months.

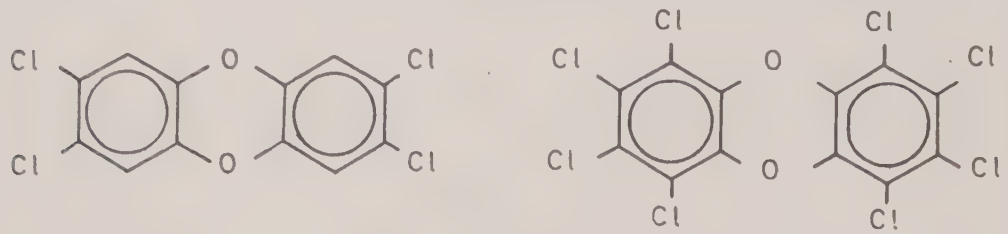


1, 2 - Dichloro-ethane | Tetrachloro-ethene | Pentachloro-ethane

Figure 4. Typical by-products from PVC manufacture

12.1.5 Chlorinated di-benzo-p-dioxins

These have recently been found to be present in many products of chlorinated phenol origin, for instance, in chlorinated phenoxy acids used as herbicides, or chlorinated phenols used as fungicides (Figure 5). This compound group is very resistant to biological and chemical attack and is fat soluble, but seems on the other hand to be rather unstable to ultraviolet light. Up to now, no significant residues of the dioxins or their metabolites have been found in nature.



2,3,7,8 - Tetrachloro-dibenzo - p - dioxin

Octachloro - dibenzo - p - dioxin

Figure 5. Chlorinated di-benzo-p-dioxins

12.1.6 Conclusions

From what is known today, a monitoring system to control the presence of chlorinated hydrocarbons in nature should include, as a first priority, DDT, its metabolites, and PCBs. These compounds are the most stable and are found abundantly. Aldrin, dieldrin and endrin are still used in many areas and might thus be included, but will on the other hand make the analysis more complicated, and more expensive. It would also be useful if some laboratories, on a bilateral scale, included a search for aliphatic chlorinated hydrocarbons and chlorinated dioxins.

12.2 Analytical methods

12.2.1 Detection, quantification

Since analytical chemistry has had to increase the sensitivity of its methods, the gas chromatograph, fitted with a highly chlorine-sensitive electron capture detector, has become the most frequently used apparatus for the final "identification" and quantification. When a mixture of many chlorinated compounds is present in a sample the chromatogramme might be very complex with obvious risks for overlapping of compounds with the same retention time. The use of double column systems minimizes this risk, but it must be remembered that gas chromatography alone can never give a complete identification but only a rather good indication. The gas chromatographic analysis can be combined with some chemical treatment which, for example, destroys one compound, but not another, and thus increases the certainty of the analysis. The only other method available which can compete with a gas chromatograph combined with an electron capture detector in sensitivity is the mass spectrometer combined with a gas chromatograph. If this instrument is set for detection of the parent ion only (or the base peak or both), it has a very good sensitivity (and in praxis this is almost equal to identification).

Quantification is usually done by one of the following methods: (a) by comparing the height of a peak with that of a standard of known amount; (b) by comparing the areas instead, calculated, for instance, by means of the peak height and the width at half the peak height, or (c) by means of an electronic integrator or a disintegrator. For fully separated peaks (c) is fairly accurate and fast; (b) works well also in cases where the peaks are at least half separated, but is somewhat time-consuming. The sensors controlling the column temperature of the gas chromatograph work fairly well, so that retention times can be held constant and therefore reference to peak heights only is accurate enough.

Identification by means of a gas chromatograph (with a good temperature controller) and an electron capture detector is the most sensitive method available. Quantification can be done by peak height comparison to a standard.

12.2.2 Extraction and clean up

In the extraction of the chlorinated hydrocarbons the fat must be extracted in amounts comparable to some official fat estimation method, for instance the so-called Torrey method (Hansson and Olley, 1963) which uses a chloroform methanol extraction; this gives higher fat values than soxhlet extraction with ether. The reason for applying special extraction methods instead of the official ones is that the latter involve the use of chlorinated solvents. Such solvents - because of their chlorine content - seriously upset the function of the electron capture detector of the gas chromatograph.

The fat content must be estimated to make it possible to refer residue values both to fresh and fat weight basis. If the fat estimation is in agreement with an official fat estimation method, it is at the same time almost certain that the chlorinated hydrocarbons are also extracted quantitatively as they are fat soluble. Before injection of the extract into the gas chromatograph all fat must be removed. Two alternatives are discussed below:

(i) Destruction method: In all cases where only lindane, DDT (and metabolites) and PCB have to be estimated, the sulphuric acid method can be used. Each 50 mg of fat is dissolved in 1 ml of hexane and 1 ml of concentrated sulphuric acid. After shaking and centrifugation the sample is pure enough to be injected into the gas chromatograph. If the resulting chromatogrammes are too complex to be evaluated, the method used by Westöö and Norén (1970) can be applied for further separation. This is necessary especially for DDD and DDT in the presence of high amounts of PCB. A good alternative to this method is described by Holden and Marsden (1969).

(ii) Non-destruction method: Dieldrin, endrin and, to some extent, also aldrin are destroyed by the sulphuric acid method. If these compounds have to be taken into account the acetonitrile or dimethyl sulphoxide/hexane partition can be used. Three partitions almost quantitatively transfer the chlorinated hydrocarbons (but not the fat) from the hexane to the acetonitrile or dimethyl sulphoxide respectively, from where it can be recovered by dilution with water and re-extraction to a new hexane phase. The extract obtained by this method is not clean enough to be injected into the gas chromatograph but has to be purified further on a partly deactivated florisil column. With this step it is also possible to separate the most polar substances, such as dieldrin, endrin, from PCB and the DDT-family.

In the method of Holden and Marsden (1969) a double column system is used, where the first is an alternative to the partition method for separation of the fat. This column is made up of alumina partly deactivated with 5% of water. The second column contains silica gel which can separate, to some extent, different classes from each other.

To make it possible to relate the residue to fat weight basis, the fat estimation has to be standardized. When only the DDT-family and PCB have to be estimated, the rapid sulphuric acid method can be used to remove fats. In other cases either partition or column clean up have to be used.

12.2.3 Analytical sensitivity

With a properly working gas chromatographic system (tritium detector) and a retention time for DDT of 20 minutes, the following amounts (in ng) can easily be detected: Lindane 0.01, aldrin 0.02, p.p´.-DDE 0.04, p.p´.-DDD 0.08, p.p´.-DDT 0.12, and dieldrin 0.05. PCB with a mean of 50% chlorine gives 14 peaks and the sum must be around 0.5. When the substances are present in these proportions the peaks are of the same height and around 50% of the scale, with a noise level of about 0.5%. This means that it is possible to see a peak approximately one-tenth of these values.

When these values have once been estimated (apparatus constants) the lower detection limit in the analytical method only depends on the sample size in relation to the final volume. In some cases therefore, the lower detection limit is dependent on the possibility to get enough sample. This is often the problem with plankton or organs from small animals. If the sample is 0.1 g, and the final volume can hardly be smaller than 0.1 ml, it means that DDT can be estimated down to 10 ng/g (ppb) and lindane down to 1 ng/g. If sea water is to be analysed, however, the reverse is the case. The sample size has no limitation, but extraction and concentration to the final volume is extremely time-consuming and difficult.

A method published by Ahling and Jensen (1970) filters a total volume of up to 200 l of water, through a silanized solid support covered with oil. The filter weighs only a few grammes, but the filtering time is about 24 hours. The adsorbed chlorinated hydrocarbons are easily eluted from the filter by means of hexane and the final volume can be made as little as 0.1 ml. This will give a lower detection limit of 0.5 ppq (parts per quadrillion, 0.001 ppb). With the more realistic volume of 100 l and a final volume of 1 ml, the detection limit is 10 ppq. It is often necessary to analyse at this level in sea water or rain water.

Recently, there have been several publications on the use of Amberlite XAD-4 as the adsorbent for the extraction of chlorinated hydrocarbons from water (Kennedy, 1973; Musty and Nickless, 1974).

12.3 References

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Hansson, S.W.F. and J. Olley, 1963 Application of the Blight and Dyer method of lipid extraction to tissue homogenates. Biochem.J., 89(3):101-2

Holden, A.V. and K. Marsden, 1969 Single-stage clean-up of animal tissue extracts for organochlorine residue analysis. J. Chromatogr., 44:481

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Musty, P.R. and G. Nickless, 1974 Use of Amberlite XAD-4 for extraction and recovery of chlorinated insecticides and polychlorinated biphenyls from water. J. Chromatogr., 89:185-90

Westöö, G. and K. Norén, 1970 Determination of organochlorine pesticides and polychlorinated biphenyls in animal foods. Acta Chem.Scand., 24:1639-44

13. THE INFRARED (IR) SPECTROPHOTOMETER AND ITS USE IN ANALYTICAL WORK

by

Stig R. Carlberg

In many respects the principal construction of the IR photometer and its use in analytical chemistry bears a strong resemblance to that of the common UV-VIS instrument. However, some differences will be pointed out here.

The IR (or heat) radiation is invisible to the human eye. Water molecules absorb IR radiation strongly. Therefore water cannot normally be used as solvent for samples. When using this photometric technique in marine research the compounds of interest must be extracted from the water, or otherwise dissolved in a suitable solvent. If spectra are to be recorded over the entire spectral range of the instrument, it is often necessary to purge the instrument with nitrogen gas in order to achieve high quality spectra. In most cases the path-lengths used in IR spectrometry are much shorter than those in UV-VIS technique, often in the order of fractions of a millimetre.

IR radiation promotes transitions in a molecule between rotational and vibrational energy levels of the ground (lowest) electronic energy state. This is in contrast to the energetically more powerful UV radiation, which facilitates transitions between vibrational and rotational energy levels of different electronic levels. Thus a UV recording shows what happens between and within electronic levels inside the atoms in the sample, whereas an IR recording shows what happens within the molecules (or their functional groups). Such groups are $-OH$, $-NH_2$, $=CO$, $\equiv CH$, $=CH_2$, $-CH_3$ and others. As these groups absorb energy at their characteristic wavelengths (frequencies) it is possible to identify them in a sample by means of the IR technique. In fact this is one of the major applications. In this way chemical compounds can be identified and their structures determined, if the sample contains just one major compound with impurities not exceeding a few percent.

By convention, band positions are quoted in units of wave number (ν), which are expressed in reciprocal centimetres (cm^{-1}), usually styled band frequencies. However, the true unit of frequency ($\bar{\nu}$) is given in reciprocal seconds (sec^{-1}). An alternative term, wavelength (λ) measured in microns (μ) is also used to indicate band position. The relation between these units is given in the expressions:

$$\nu = \frac{1}{\lambda} \; ; \; \bar{\nu} = \frac{c}{\lambda} \qquad (c = \text{velocity of light})$$

$1\mu = 10^{-4}$ cm $= 10^4$ Å; hence $10\mu = 1\,000$ cm^{-1} and $2.5\mu = 4\,000$ cm^{-1}.

As the absorption at any given wavelength is proportional to the concentration of the functional group, Lambert-Beer's law is valid. Thus calibration and quantitative measurements can be performed as was described for the common photometric methods in heavy metal analyses.

A schematic description of an instrument, which can work either in a double-beam or single-beam mode is presented in Figure 1.

In the double-beam, optical null mode a nichrome wire source emits stable IR radiation that is split in two beams. One beam is directed through the sample and the other beam is directed through the reference in the sample compartment. Upon leaving the sample compartment, each beam is directed to a beam alternater mechanism which consists of a two-lobed mirror that rotates, to alternately direct the two beams along their respective paths at ten cycles per second.

From the beam alternater mirror, the beams are directed along an identical path in alternate pulses of energy that are filtered by one of six long-pass interference filters which transmit only specific regions of the spectrum.

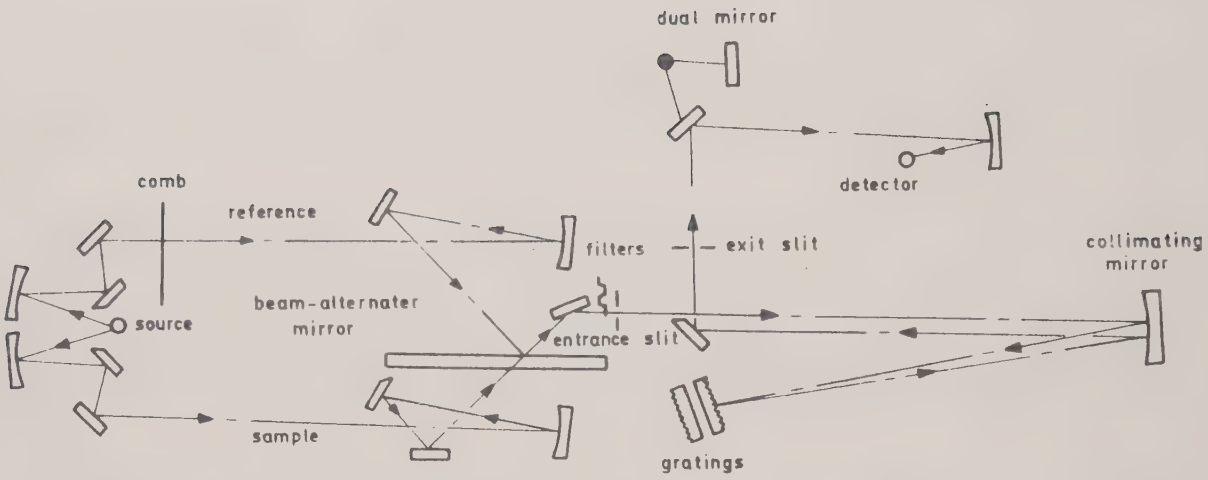


Figure 1. Principal layout of an IR spectrophotometer (Beckman IR-10)

The transmitted beams of radiant energy are passed through the entrance slit, into the monochromator, and are reflected from a collimating mirror onto one of two precision replica diffraction gratings. The grating disperses the incident radiation, which is reflected back to the collimating mirror. Dispersed radiation is focused on the exit slit. As the grating rotates, one narrow band of radiation emerges from the exit slit and is focused on the thermocouple detector.

When the sample and reference beams are of equal intensity, no signal is generated in the detector system. However, when the sample absorbs energy, the beams are of unequal intensity; this generates a signal in the detector system that is proportional to the difference in energy of the two beams. In response to this signal an optical attenuator, or comb, is moved into the reference beam to decrease reference energy until beam balance (optical null) is restored. Since the recorder pen is coupled mechanically to the comb, and the null position of the comb is a direct indication of transmittance, the pen records on the chart paper a spectrum of sample transmittance versus wavelength or wavenumber.

The single-beam, electrical null mode will not be considered here, as it will not be used for the analyses described in this manual.

References

Cross, A.D., Introduction to practical infrared spectroscopy. London, Butterworths, 86 p. 1964

Silverstein, R.M. and G.C. Bassler, Spectrometric identification of organic compounds. 1967 New York, John Wiley, 256 p.

In the text have been cited a few lines from the book by Cross, describing the differences between ultraviolet and infrared radiant energies. The material was used with the kind permission of the publishers, Butterworths (London).

The figure describing the optical system of the photometer IR-10 and the text decribing its function was used with the kind permission of Beckman Instruments, Inc.

14. ANALYSIS OF AQUATIC MINERAL OIL POLLUTION A PRESENTATION OF ALTERNATIVE ANALYTICAL TECHNIQUES

by

Stig R. Carlberg

14.1 Introduction

A number of different analytical methods have been worked out for investigations of aquatic pollution caused by mineral oil. Not all of these methods are useful for routine work, but those today considered good all rely on chromatographic and/or spectroscopic techniques.

Because mineral oils all consist of a very large number of different components with varying properties, it has not been possible to design one analytical method which is both specific and sensitive while simple to perform as routine work with inexpensive equipment. Consequently, in this chapter four different techniques are described; two purely chromatographic ones, one spectroscopic and one which is a combination of these two basic principles. All the methods have their advantages and disadvantages. It should be remembered, however, that in pollution studies and monitoring programmes it may be a good idea to use a method which offers a fair sensitivity and the possibility of analysing a large number of samples, even if the results are not entirely specific. Such a method can be combined with a more time-consuming one for analysis of a smaller number of samples taken at random or samples from a few sampling stations, or samples with a concentration exceeding an accepted limit.

14.2 Thin layer chromatography (TLC) technique

The basic concept of TLC - which is a liquid solid adsorption chromatography - is described in chapter 11.

A sensitive method (capable of detecting down to fractions of one milligramme) is described by Giebler et al. (1964). They used glass plates coated with a mixture of equal parts of aluminium oxide and silica gel. The oil had been extracted from the water samples with carbon tetrachloride (pentane or similar may be used) and was applied as some µl from solutions containing 1 mg/ml. The plates were developed in hexane, and if the plates were finally sprayed with molybdatophosphoric acid in butyl alcohol, it was possible to identify mineral oils not only from their R_f-values but also from the colour of their spots, which differed from those containing fatty material. Another interesting variant is that described by Beynon et al. (1972). They coated their plates with silica gel and developed them in chloroform. The main feature of this variant, however, was the quantification step. The usual way is to estimate the area of the spot or spots from a sample, but this estimation is difficult to do with a great degree of accuracy. This step was somewhat simplified by grooving channels of uniform width in the coated layer of the plate (Figure 1). Thus, with a constant width of the spot, only the length of it has to be measured and the area of the spot is determined with better accuracy. This quantification is coarse and the authors pointed out that for better accuracy and precision, standards should be run on every plate for control of the variation of the surface areas of the calibration standards. The spots were visualized with iodine vapour, but of course it is here also possible to spray with molybdatophosphoric acid as described above. The method was said to be limited to oil components boiling above 150°C, when the samples were extracted with carbon tetrachloride. The sensitivity was reported as 0.5 mg/l using 1-l samples.

Once the technique is mastered, these methods are easy, fast to perform and offer a good sensitivity. It is possible to differentiate between mineral oil and other extracted substances. Differentiation between mineral oils of various types such as light fuel oil and lubricating oil or tar oil is possible to some extent, whereas it is impossible to tell between two oils of the same type (e.g., fuel oil No. 1) from two different sources. The

methods may be modified to meet special requirements. Stahl (1965) gives suggestions on these as well as a detailed description of the TLC technique in general.

14.3 Gas liquid chromatography (GLC) technique

The basic concepts of this technique and related equipment are also found in chapter 11. As for the other techniques, the oil must first be extracted from the water using a suitable solvent such as carbon tetrachloride, hexane or equivalent. If the oil pollution appears as slicks floating on the water or lumps on beaches it is sufficient just to dissolve a few milligrammes of material per millilitre of solvent.

As for all GLC analyses, the choice of stationary phase in the column is essential. The most common phases of all those giving sufficient resolution of the components in oil are the silicones, Apiezon L, SE-30 and OV-1. These phases are obtainable from all suppliers of GLC equipment. They may be purchased either as fabricated columns ready for mounting in the chromatograph, or as pure substances for column preparation in the analysing laboratory. For further details, the reader is referred to the vast literature in this field (Beynon et al., 1972; Brunnock et al., 1968; Duckworth, 1970; Ehrhardt, 1972; McNair, 1968; Pattison, 1969; Ramsdale and Wilkinson, 1968).

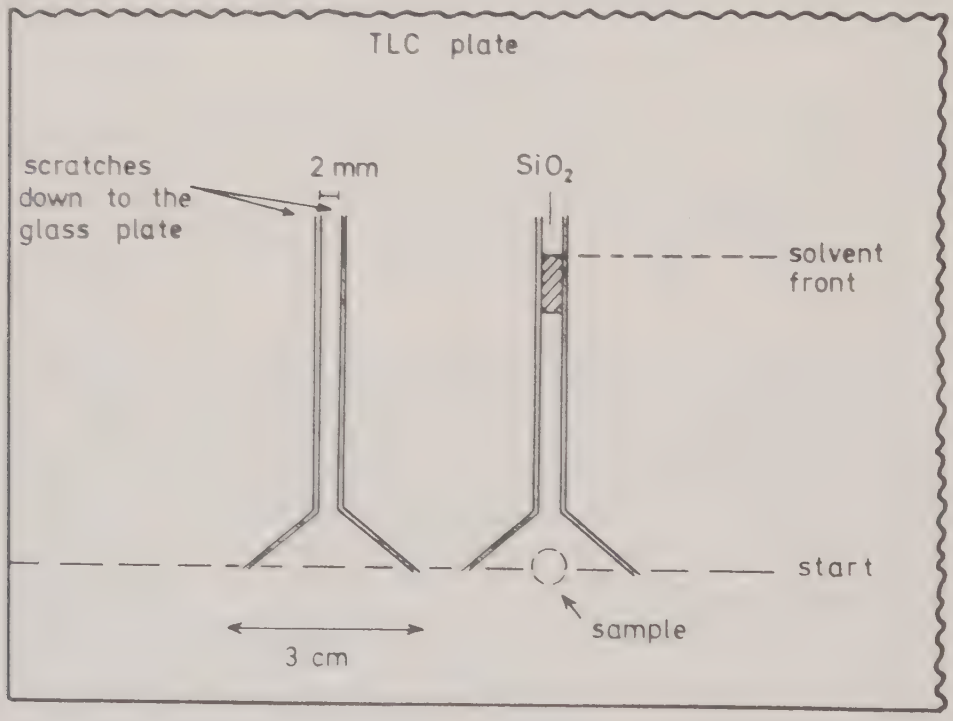
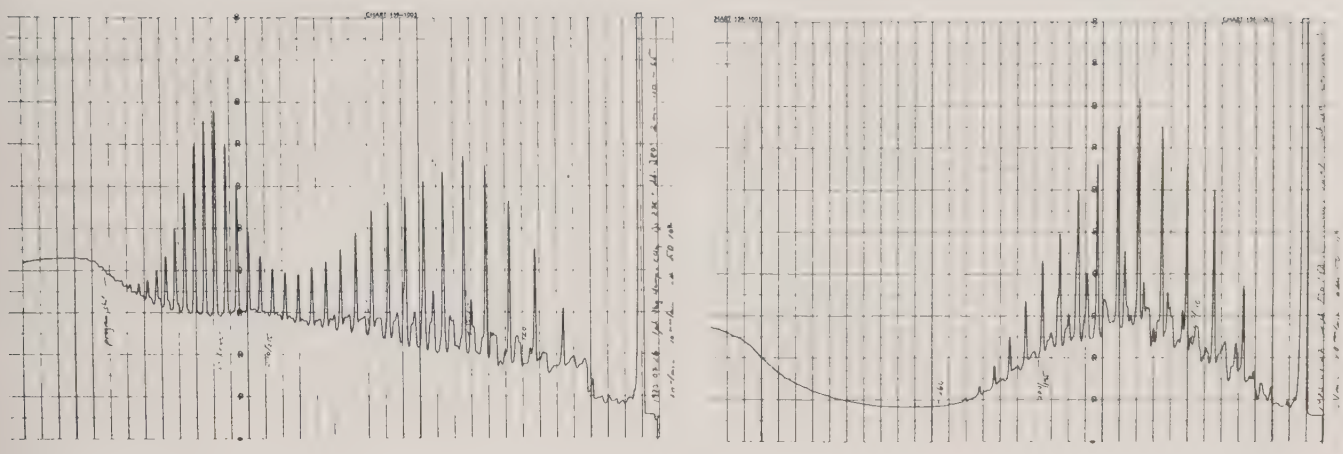
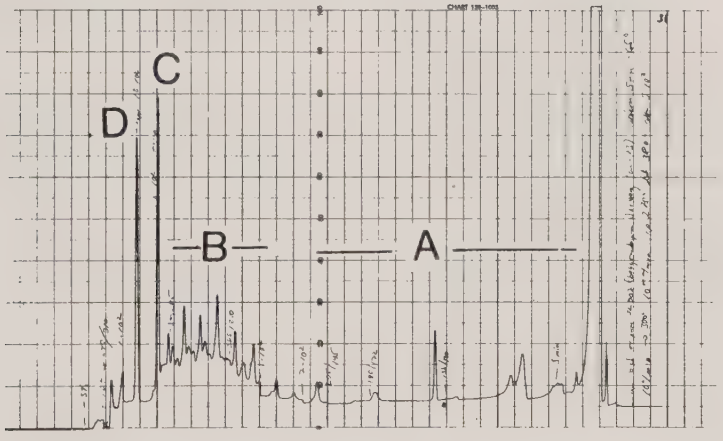


Figure 1 Channel thin layer chromatography

As the GLC gives a peak on the recorder for every component detected in the sample - provided that the separation is complete - this technique is a very good means for identification and characterization. As mineral oils contain a great number of components, the resulting chromatogram always shows many peaks even if the separation is incomplete. Thus the interpretation of the results obtained is rendered difficult. In most cases a complete identification of all components is not necessary as different oils give different (and often very characteristic) chromatograms. Those chromatograms, in turn, are of quite other appearance than those of the natural organic substances in sea water (Figure 2).



a b



c

Figure 2 Gas chromatogram of a heavy fuel oil (a) and a light fuel oil (b). The heavy oil contains more components (more peaks) and has a wider boiling range than the light fuel oil. All parts of these chromatograms are recorded in one and the same scale.

A chromatogram of the natural background of organic substances in a water sample is shown in (c). (However, the region B shows slight influence of a fuel oil). Here the different parts are recorded in different scales, in order to keep the peaks within the range of the paper. If the peaks in region B are taken as reference, peak C and those in region A should be 5 times higher and peak D should be 10 times higher than shown in the figure. The chromatogram is dominated by three peaks: D, C and one in region A. This corresponds well with other findings about organic products, which can be extracted from phytoplankton.

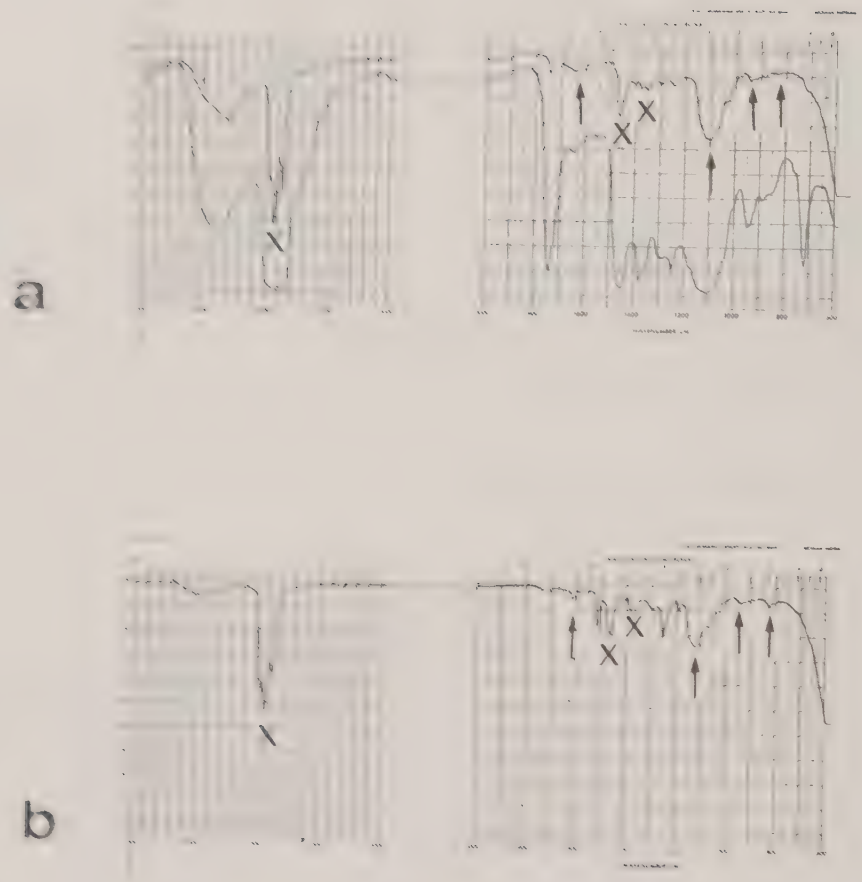


Figure 3 (a) Infra-red spectrum of a petroleum-based dispersing agent used to clean up water and shore areas after oil-spillages. The sample was analysed at two different concentrations. The petroleum peaks are cross-marked. The peaks, which are indicated by arrows are representative for the emulsifier (a surface active agent of non-ionic type):

1240–1245 cm^{-1} (a small band) from alkyl phenol

1100–1120 cm^{-1} (a strong band from ether bonds) non-ionic surface active agents in general

950 cm^{-1} (and sometimes also 830–850 cm^{-1}) moderate or weak bands from the ethyleneoxide chain of the surface active agent

(b) Infra-red spectrum of an extract of a water sample, which was collected after a dispersing operation. Petroleum peaks are cross-marked. The peaks caused by the emulsifier described above are marked with arrows.

The GLC technique is very sensitive, requiring sample volumes of only a few µl if the sample extract contains some mg of substance per ml. Thus, if the water sample under investigation is heavily polluted by oil, a suitable part of the extract can be injected directly. If on the other hand the oil concentration is low, or if the sample just contains the natural background of substances, the extract will most probably have to be concentrated 10 to 100 fold or even more.

Because of the wide boiling range of mineral oils it is not possible to run the GLC analysis at a fixed temperature. To keep the analysis time to a minimum, temperature programming must be used. This means that the temperature in the column is raised at a known and reproducible rate in order to first obtain sufficient separation between the low boiling components and then elute the highest boiling components within an agreeable time. The starting temperature is governed by the lower limit of the boiling range of the sample. The programming rate (^{o}C/minute) is governed by the number of components within a certain boiling range, the resolving capacity of the column and finally the maximum temperature is limited by the maximum temperature tolerance of the stationary phase in the column. The figures given in the paragraph below are examples of a programmed GLC analysis. After injection of the sample, the temperature of the analytical column is kept constant (at 50^{o}C) for some minutes, then the temperature is increased at a fixed rate (10^{o}C/min) up to a final temperature of 300-350^{o}C. As the strip chart recorder advances the paper with a constant speed, the distance in the recording between injection and a peak is a measure of the retention time of the component and also of the temperature at which the component was eluted. The area of the peak is proportional to the amount of the substance. Thus it is also possible to make a quantitative estimation of each component, but this requires separate calibrations with standards as the peak area corresponding to a given amount is not the same for all substances.

From the analytical column used, the components are eluted in the order of increasing boiling points. On comparing with test components of known boiling points, it is possible to determine the boiling range of the polluting oil. It is also possible to estimate if the dominating part of the oil is high boiling or low boiling.

As mentioned above, it is possible to perform quantitative analysis. However, this is rendered difficult by the fact that every sample produces so many peaks. Quantification can be more easily made by another method, for instance, the photometric techniques or by weighing the extracted material (after evaporation of the solvent) on a microbalance.

For further descriptions of the possibilities of identifying oils by GLC the reader is advised to read the extensive literature in this field. Some pertinent references are found in the reference list.

14.4 Infra-red (IR) spectroscopy

The IR spectrophotometer and the basic principles of this technique are described in chapter 13 "The infra-red (IR) spectrophotometer and its use in analytical work".

The analytical method described in chapter 17.4 is based on extraction of the oil from the water by carbon tetrachloride, clean-up of the extract by column chromatography and subsequent measurement of the infra-red absorption of the extract.

The method is sensitive - 0.05 mg/l is the detection limit. However, it should be remembered that this technique is non-specific, as the sample consists of a complex mixture of substances. What is extracted by the carbon tetrachloride is a mixture of "polar" and "non-polar" substances. Most of the components in a mineral oil are of "non-polar" character and consequently the analysis is focused upon these. To remove the "polar" hydrocarbons, the sample is chromatographed on a small column filled with aluminium oxide (liquid-solid adsorption chromatography). The "non-polar" hydrocarbons pass the column and their total concentration is measured by infra-red photometry.

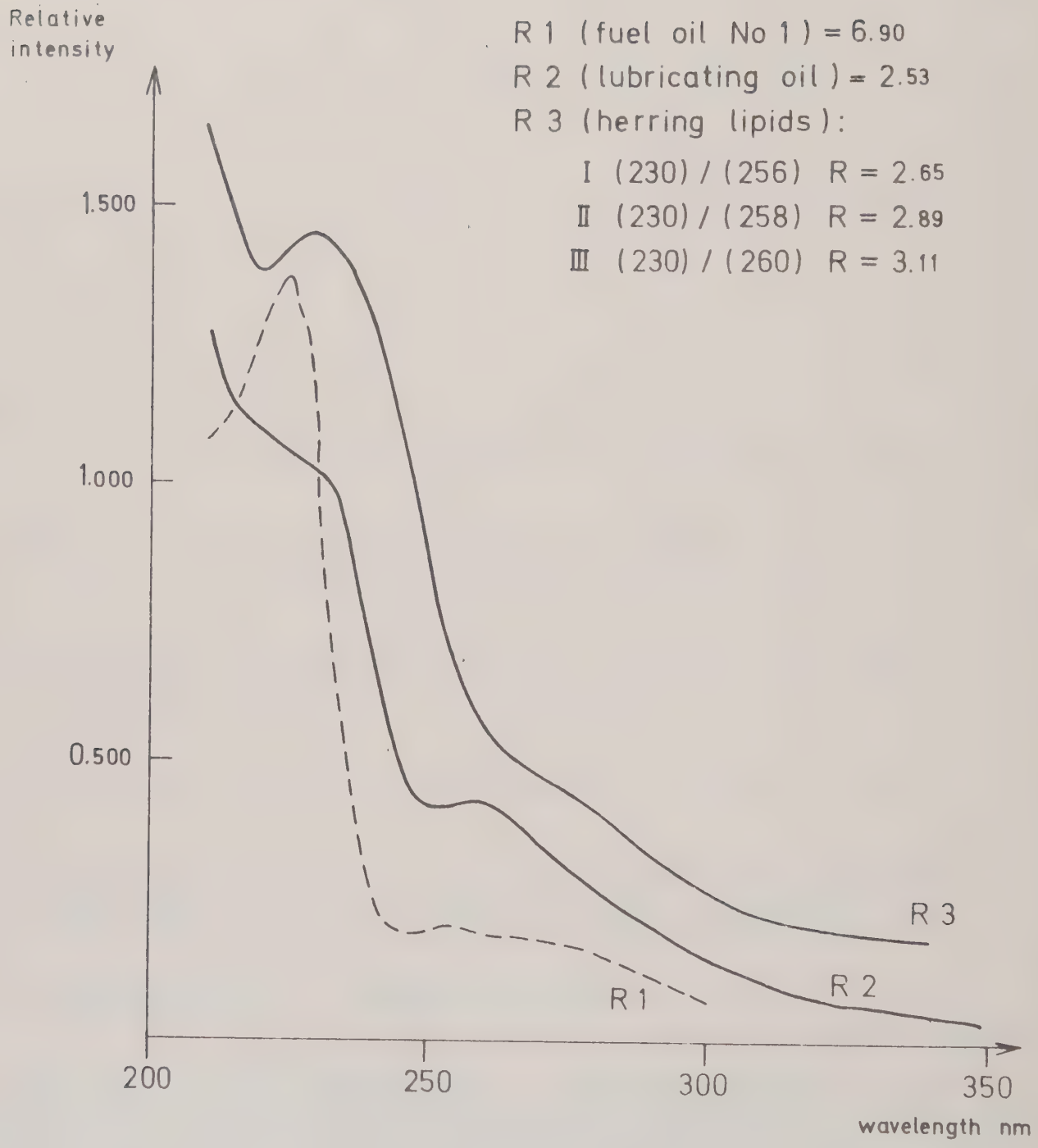


Figure 4 UV-absorption spectra for various oils

The words "polar" and "non-polar" are put here in quotation marks because they are not exactly in agreement with the common meaning of these terms. In this method the substances passing the column are termed "non-polar", whereas those retained by the column are termed "polar". Thus, for instance, the aromatic substance benzene (which is non-polar) passes the column almost completely but the non-polar aromatic phenantrene is quantitatively retained by the column and thus termed "polar".

What is actually measured is the spectral region between 3-4 µm, where the functional groups $\equiv CH$, $=CH_2$ and $-CH_3$ absorb radiation. This absorption by functional groups results from the total amount of each group in all molecules of different substances in the sample. Thus the concept "mineral oil" has to be redefined in terms of infra-red spectroscopy: "Hydrocarbons - all substances containing CH-, CH_2- or CH_3-groups, no matter whether they are pure hydrocarbons or hydrocarbon derivatives of some kind, which are not retained by aluminium oxide and which show infra-red absorption bands at 3.38 µm (2 959 cm^{-1}) and 3.42 µm (2 924 cm^{-1})".

The method is not capable of distinguishing between "non-polar" hydrocarbons from mineral oil and those from other sources, such as marine life. However, the concentrations of the latter products seem to be very low.

For calibration of this method - as well as for the others - it should of course be preferable to use the same oil as is present in the samples. However, as it is seldom known which oil is present, a good compromise is offered in the form of a standard mixture consisting of n-hexadecane (37.5 percent by volume), i-octane (37.5 percent) and benzene (25 percent).

The usefulness of this standard mixture was demonstrated when analysing some industrial effluents, which contained high concentrations of mineral oil. Before the infra-red measurements, the extracts had to be diluted 100 fold. Thus it was possible to carefully evaporate the solvent and weigh the residues for comparison with the spectroscopic method. The results obtained are shown in the table below. For further details of the calibration see chapter 17.4.

Industry	Oil mg/l IR-spectroscopy	Oil mg/l gravimetric
A	324	319
B	138	140
C	142	142

These concentrations are of course much higher than those normally encountered in water pollution. Nevertheless it has been demonstrated that the standard mixture is in good agreement with most kinds of mineral oil (Scholl and Fuchs, 1968).

If enough sample is available (industrial effluents, floating oil slicks or oil lumps found on the shores) it is possible to run IR spectra in the entire spectral region of the photometer. The sample is deposited as a thin film (some hundredths of a millimetre) by smearing as a paste or evaporation from a solution onto an alkali salt disc (NaCl, KBr). This offers additional means for differentiation between different oil samples (Figure 3).

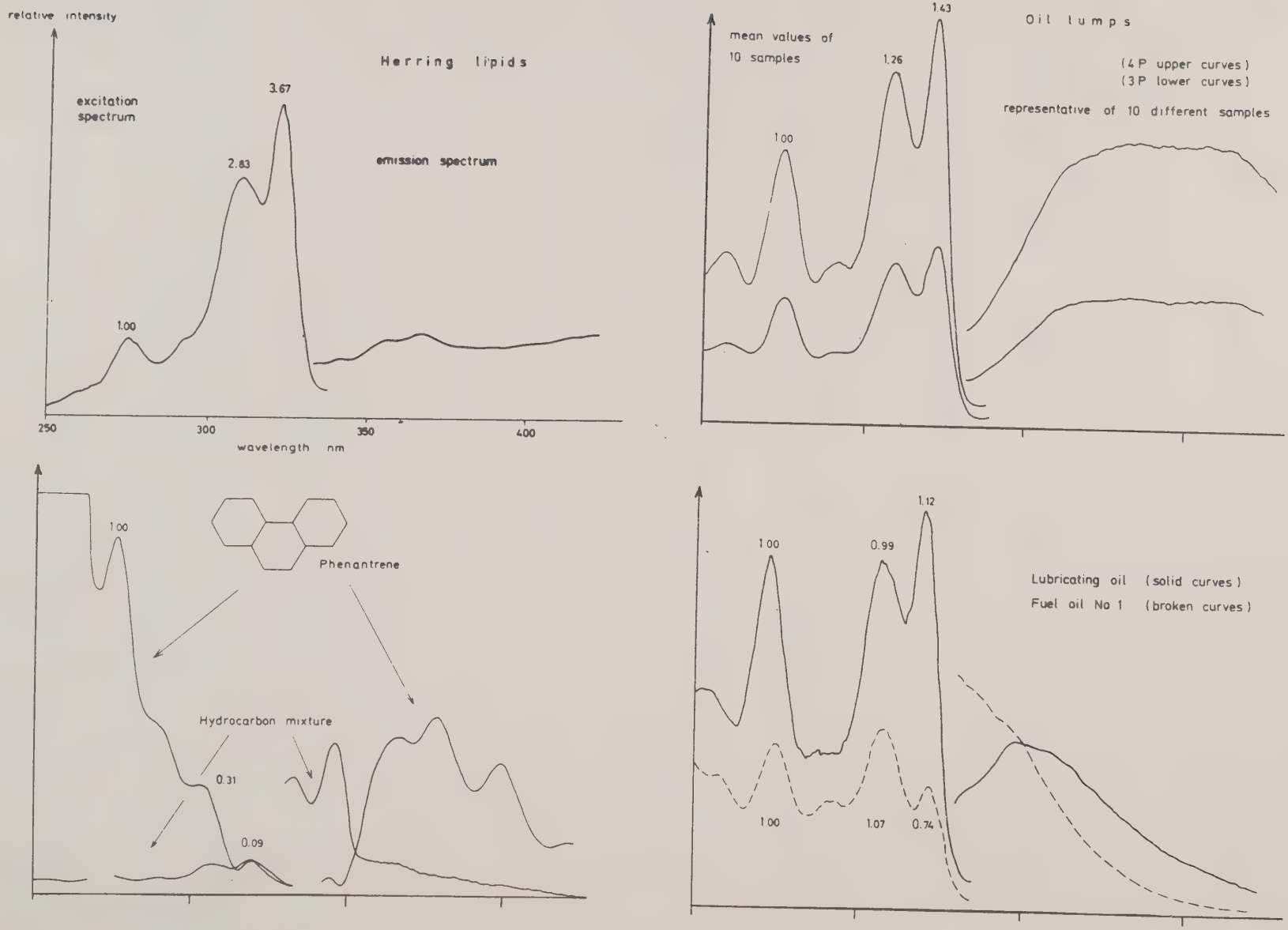


Figure 5 Ultra-violet fluorescence spectra

14.5 Ultra-violet (UV) absorption spectroscopy

In contrast to the IR method where the aromatic substances were excluded, this technique concentrates on these substances. Thus the two spectroscopic techniques are good complements to one another. As aromatic substances are rarely produced in the marine environment, their detection may be a good criterion of pollution. A sensitive method, detecting down to 0.01 mg/l, has been published by Levy (1972). It is simple to perform; the sample is extracted with any suitable solvent such as chloroform, carbon tetrachloride, hexane or equivalent. The absorption of the extracted material is then measured between 210 and 350 nm. Hexane is one of the few solvents which are transparent at the shorter wave-length, so if it is not used for the extraction, a change of solvent has to be done before the measurements are made. Preferably, a double-beam scanning photometer is used to save time and achieve the highest accuracy. If such an instrument is not available, manual readings have to be made at every 5 nm between 210 and 350 nm (between 220-230 nm and 250-260 nm at every 2 nm).

From the resulting graph it is possible to differentiate between oils of various kinds. The maximum absorption value in the region 220-230 nm is divided by that in the 250-260 nm region. This R-number is a characteristic for every type of oil. For details see Figure 4. For residual oils (oil lumps) the R-number varies between about 1.20-1.60, for lubricating oil 2.50-4.00, and refined products such as fuel oils have very high values - 6 or even more. However, lipid extract from herring showed an R-number similar to that of lubricating oils. This indicates a disadvantage of the method which can be overcome by running the sample once again after chromatography as this removes the fatty matter completely, whereas the aromatic compounds are removed only to some extent. The absorption value at any of the two maxima can be calibrated with known concentrations of oil in order to make quantitative analysis possible.

The main features of this method are that it is sensitive and simple to perform, which makes analyses of a large number of samples possible. As was pointed out by Levy, the R-numbers seemed to be constant for more than one year for a given oil. This of course facilitates the work of differentiation between various oils.

14.6 Ultra-violet fluorescence spectroscopy

For this technique, a procedure described by Keizer and Gordon (1973) is discussed. Their analytical method is applicable to oil lumps as well as water samples. In the latter case the samples are extracted in the same way as described for the UV absorption technique. The fluorescence technique is very sensitive, a detection limit of 1 µg per litre is claimed by the authors.

The results are presented in Figure 5, which consists of four pictures. All spectra were obtained in the same way. The samples were dissolved in hexane and an Aminco-Bowman spectrophotofluorometer was used. The instrument was equipped with a xenon-mercury lamp. When recording the emission spectra (right side of each picture) the excitation monochromator was set at 313 nm and when recording the excitation spectra (left side of each picture) the emission monochromator was set at 380 nm.

In the top left picture, the spectra from herring lipids are shown. The emission spectra show nothing of interest, but in the excitation spectra three peaks are clearly visible. If the intensity of the smallest peak is defined as 1.00, the other two have the values 2.83 and 3.67 respectively. This should be compared with the excitation spectra of some oil lumps which are found in the top right picture. Here only two spectra are shown, but they are representative for ten samples. These spectra are dominated by the same three peaks found in the lipids, but the general appearance is different. The relations between the peaks are 1.00 - 1.26 - 1.43. These figures are mean values of single analysis of the ten samples. The variations were very small; 1.24 - 1.30 and 1.38 - 1.48 for the two highest peaks.

The excitation spectra obtained in this way do not offer any possibilities for differentiation between samples of the same nature. However, as has been shown by other authors (Freegarde et al., 1971; Thruston and Knight, 1971) a number of the instrumental parameters may be changed and thus further information can be obtained. The application of fluorescence techniques to oil analysis is comparatively new, and many laboratories seem to be involved in work aimed at developing the procedures further.

The emission spectra of the oil lumps do not provide much useful information. We can only note that with increasing wave-length the intensity goes up at first and then levels out. This should then be compared with the emission spectra obtained from the fuel and lubricating oils. These are found in the bottom right picture. In these spectra the intensity decreases rapidly with increasing wave-length after 350 nm. This is of course an obvious difference but the excitation spectra are more informative, as they can easily be described in figures. These spectra are also dominated by the same three peaks discussed earlier. The two oils give spectra which are not like one another and furthermore they are quite different from those of the oil lumps. Expressed in figures the fuel oil is 1.00 - 1.07 - 0.74 and the lubricating oil 1.00 - 0.99 - 1.12. On the other hand, as these values are based on the analysis of just one oil of each type, one cannot claim that they are entirely representative for their groups.

Finally, in the bottom left picture of Figure 5 the spectra of some references are presented. Phenantrene is an aromatic substance which may be found in several oils. The emission spectrum shows some peaks, and so does also the spectrum of the hydrocarbon mixture. This mixture is the same as that used for the calibration of the IR photometer when performing quantitative analysis.

When analysing all these samples and references, it is striking to notice that great amounts of the hydrocarbon mixture and of the herring lipids are required to obtain the spectra, in comparison to those minute amounts required of the different petroleum products. The excitation spectrum of the hydrocarbon mixture is without interest. The excitation spectrum of the phenantrene is quite impressive. At the lowest wave-lengths the intensity is very high and at 275 nm and further the three well known peaks are found but this time in a descending order: 1.00 - 0.31 - 0.09.

It can be concluded that the fluorescence techniques - especially the excitation - are very sensitive and capable of distinguishing between different types of oils and also between oils and lipid matter. The possibilities to distinguish between different samples of the same type seem to be less good, when the technique is applied as was done here. However, the instrumental variations are many and not yet fully investigated.

14.7 Advantages and disadvantages of the TLC, GLC, IR and UV techniques

14.7.1 TLC

(+) Very inexpensive equipment. Complete basic kits are commercially available for about U.S.$ 300-400. Quite easy to perform when the technique has been learnt. To some extent quantitative analysis is possible as well as characterization and differentiation.

(-) Not so sensitive as the other techniques. 1 mg/l or somewhat better could be expected in routine work. The technique requires some skill and is slightly more difficult to learn than the IR technique.

14.7.2 GLC

(+) The most sensitive technique of the five cited here. Provides very good possibilities for characterization and differentiation. Quantitative analysis is possible.

(-) Expensive equipment, U.S.$ 4 000- 5 000 or more. Of the five techniques discussed this one is the most difficult to learn and also to apply, requiring meticulous care. The analytical columns may be deteriorated by high boiling components from the samples.

14.7.3 IR

(+) Sensitive - 0.05 mg/l can be obtained in routine work by extracting two litres of water sample. Applied in this way, the IR and also the UV technique are the easiest to perform. If the sample is applied as a film or "paste" on an alkali salt disc, the technique offers possibilities of characterization and differentiation.

(-) The equipment is rather expensive, U.S.$ 5 000-6 000 or more. When applied to extracts the technique offers small possibilities of differentiation between sample types. A combination with other methods is recommended in this respect, however, see also Mattson et al. (1972).

14.7.4 UV - absorption

(+) This is a very sensitive technique - 0.01 mg/l is claimed by Levy (1972). The procedure is easy to apply and if a scanning double beam instrument with recorder is provided, it is possible to analyse many samples in a relatively short time. Good possibilities to characterize and differentiate between oils of different types and even between the same type of oil from different sources (Levy, 1972).

(-) The technique concentrates upon the aromatic components in the sample and is comparatively insensitive to the alkanes, olefines and other types of substances in the mineral oils, thus being a contrast to the IR technique. The equipment is rather expensive. A double beam photometer with scanning facilities costs about U.S.$ 4 000-5 000. On the other hand, such an instrument is also capable of spectroscopic work in the visual region of the spectrum, thus being suitable for all nutrient analyses, etc., in the laboratory. From that point of view, the UV photometer is not such an "exclusive" investment as the IR photometer.

14.7.5 UV - fluorescence

(+) The second most sensitive technique of those discussed here, giving very good possibilities for characterization and probably also for differentiation between samples of various origin. The procedure is easy to learn and also to apply as a routine, giving rapid analysis. Further refinements are being developed, probably making the technique more attractive in the future. With some precautions the technique is suitable for routine work, qualitative as well as quantitative.

(-) As the technique is very sensitive, it is easily upset. Very pure solvents are required as well as careful handling of sample solutions, cuvettes, etc. The instrument is expensive and may today be regarded as rather exclusive.

Note: The prices quoted above are very relative, giving the approximate cost of the equipment as it can be bought in Sweden and with the local prices recalculated into U.S. dollars. The figures are given merely to show the difference in complexity of the equipment used.

14.8 Other means of characterization

Crude oils and their refined products always contain certain amounts of heavy metals such as vanadium, nickel, copper, iron, zinc and others. The concentrations of the metals and the ratio between some of them (e.g., vanadium/nickel ratio) varies with the origin of the oil. Thus the heavy metals can be used as a complementary means of characterization and identification, provided that enough sample can be obtained (floating oil slicks or lumps, pollution on beaches, etc.). See also Stuart and Branch (1970) and Duckworth (1970).

The analysis of heavy metals in mineral oils is quite similar to that for water samples, when the atomic absorption spectroscopy (AAS) is used. The methods are discussed in chapter 9.

The combination of different detectors in the GLC technique offers new possibilities for the characterization of mineral oils. Adlard et al. (1972) used a flame photometric detector in conjunction with the flame ionization detector for specific determination of the sulphur-containing components in samples of polluting oils.

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SECTION II - PRACTICAL GUIDE TO MEASUREMENT METHODS

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15. METHODS FOR THE DETERMINATION OF PHYSICAL PARAMETERS FROM A RESEARCH VESSEL

15.1 Instruments for the determination of temperature

15.1.1 Protected reversing thermometers (PRT)

PRTs were first introduced by Negretti and Zambra (London). Since this firm stopped making thermometers in 1970, the Fishery Board of Sweden has bought PRTs from Kurt Gohla, Kiel, Federal Republic of Germany; they are also manufactured by several other companies, e.g., Richter und Wiese, Berlin, Federal Republic of Germany.

PRTs are usually mounted on the water sampling bottles (see 15.3). An RT is sent down to the required depth in the set position (Figure 1), in which it consists of a large reservoir of mercury connected by means of a fine capillary to a smaller bulb at the upper end. Just above the reservoir the capillary is constricted and branched (with a small arm) and above this the thermometer tube is bent in a loop, from which it continues straight and terminates in the smaller bulb. The RT is so constructed that in the set position mercury fills the reservoir, the capillary and part of the bulb. The amount of mercury above the constriction depends upon the temperature, and when the RT is reversed, by turning through 180 degrees, the mercury column breaks at the point of constriction and runs down, filling the bulb and part of the graduated capillary. The loop in the capillary is designed to trap any mercury that is forced past the constriction if the temperature is raised after the RT has been reversed.

In order to correct the reading for the changes resulting from differences between temperature at reversal and the surrounding temperature at the time of reading, a small thermometer, known as the auxiliary thermometer (AT), is mounted alongside the RT. RT and AT are enclosed in a heavy glass tube that is partially evacuated except for the portion surrounding the reservoir of RT, and this part is filled with mercury to serve as a thermal conductor between the surroundings and the reservoir. Besides protecting from damage, the tube is an essential part of the device, because it eliminates the effect of hydrostatic pressure.

The following equation is commonly used for correction:

$T = T' + \Delta T$ where

$$\Delta T = \frac{(T' - t)(T' + Vo)}{K - 100} + I$$

T = the corrected temperature of the RT

T' = uncorrected reading of RT

t = the temperature (from AT) at which RT is read

Vo = the volume of the small bulb and of the capillary up to $0^{\circ}C$ graduation expressed in terms of degree units of the capillary

K = a constant, depending upon the relative thermal expansion of mercury and the type of glass used in the thermometer. For most reversing thermometers K = 6100

I = the individual calibration (e.g., from the certificate) correction of RT depending upon T'

Instead of calculating according to the given formula for every temperature correction, a handy graph (Theisen, 1947) can be used, saving considerable time (Figure 2). It should be noted that not only the graph but also its plotting paper must be prepared for each individual thermometer as described by Theisen.

15.1.2 Mechanical bathythermograph (BT)

The BT is an instrument for obtaining a record of the vertical temperature variations of sea water at moderate depths. The BT is lowered into the sea and retrieved by means of a wire rope. It can be operated while the ship is under way, at speeds of up to 12 knots.

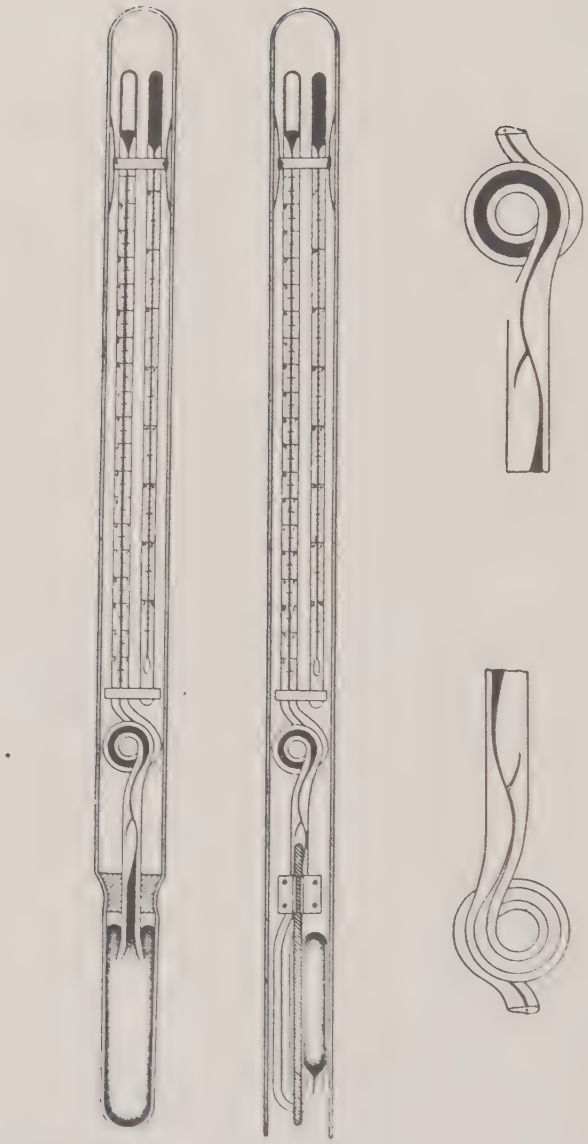

Figure 1. Protected and unprotected reversing thermometers in set position, that is, before reversal. To the right is shown the constricted part of the capillary in set and reversed positions

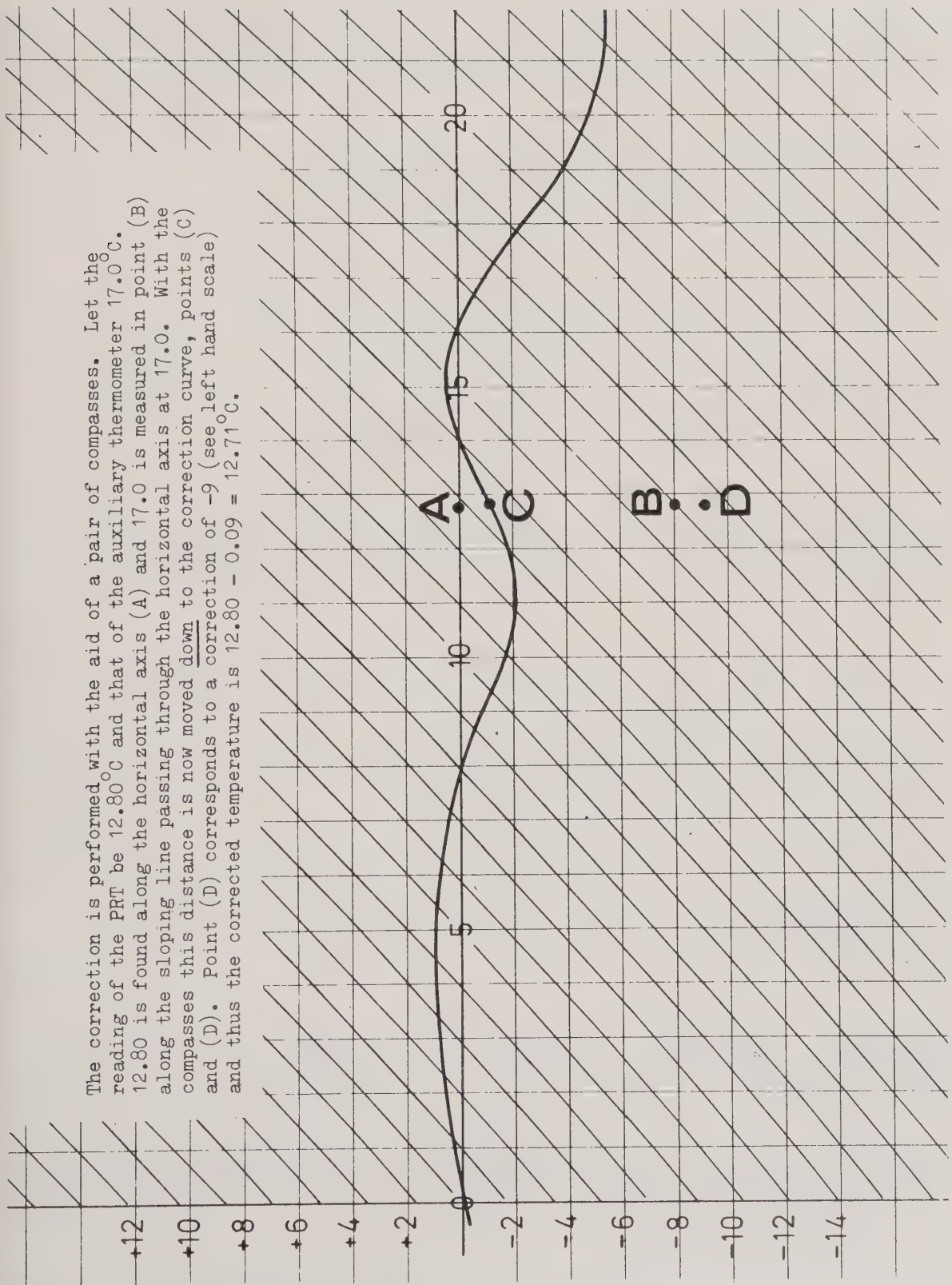
+12
+10
+8
+6
+4
+2
0
-2
-4
-6
-8
-10
-12
-14
5
10
15
20
A
C
B
D
The correction is performed with the aid of a pair of compasses. Let the reading of the PRT be 12.80°C and that of the auxiliary thermometer 17.0°C. 12.80 is found along the horizontal axis (A) and 17.0 is measured in point (B) along the sloping line passing through the horizontal axis at 17.0. With the compasses this distance is now moved down to the correction curve, points (C) and (D). Point (D) corresponds to a correction of -9 (see left hand scale) and thus the corrected temperature is 12.80 - 0.09 = 12.71°C.

Figure 2. Example of a correction chart for reversing thermometers

Phys param 4

The thermal element of the BT consists of about 15 m of fine copper tubing filled with xylene. As the xylene expands or contracts a Bourdon-type element is activated; the element carries a stylus at its free end. The stylus records, on a coated glass slide, the movements of the Bourdon tube. Slides used to be coated with soot, but nowadays, a thin gold layer is preferred. The slide is held rigid by the depth element assembly which is at the end of a coil spring enclosed in a copper bellows or siphon. The temperature range of the BT is -2° to $+32^{\circ}$C.

Water pressure, which increases with depth, compresses the siphon as the BT sinks. This pulls the slide toward the nose of the BT, at right angles to the direction in which the stylus moves; thus the trace scratched on the coated surface of the glass slide is a combined record of temperature and depth. The depth range is stamped on the nose of the BT. It is usually either 60, 135 or 275 m.

If the BT does not have a built-in swivel, include one. Connect the lowering wire thimble to the BT swivel with a shackle. Slide the BT sleeve forward toward the BT nose; this will uncover the stylus assembly and slide holder. The edge of the slide with the bevelled corner goes in first with the bevel toward the nose of the BT. Push the slide all the way in. Occasionally, check the grooves of the slide holder to make sure they are clean and free of glass chips. Move the sleeve back to cover the opening prior to putting the BT over the side of the ship. This will bring the stylus into contact with the glass slide.

Lower the BT into the water to such a depth that it rides smoothly just below the surface. Set the counter on the winch to zero. Now the instrument is equilibrated at the sea surface temperature. Then lower the BT. Do not allow it to touch the sea bottom. When the BT is again on board move the sleeve forward toward the BT nose to lift the stylus off the slide. Partially eject the slide by pushing against its edge with a pencil through the slide-ejecting part. Grip the slide carefully with the thumb and forefinger, holding it only by the edges.

Never let the temperature of the BT exceed 40°C. Suitable protection of the thermal element from hot sun, for example, can be afforded with wet cloths. After survey operations the BT should be rinsed with fresh water.

As soon as the slide is removed from the BT examine it to be sure that a suitable trace has been obtained. Label the BT slide with a sharp instrument or pencil, giving ship's name/consecutive slide number within year/year/BT instrument number.

Clean the grid (individual for each instrument) with a cloth or tissue. Place the slide in the viewer with the coated surface toward the grid and the bevelled edge toward the set screw (to remove the slide from the grid, depress the spring to loosen the slide from the grid mount). When reading the BT trace include sufficient inflexion (flexure) points to describe the temperature structure.

15.1.3 Sea surface temperature

A bucket temperature can be taken by collecting a surface sample of about 5 to 10 litres in any suitable container. Samples taken from a vessel must be obtained as far away as possible from any discharge outlets from the hull, and if the vessel is under way they should be taken near the bow so as to avoid the churned-up water of the wake. The bucket should be filled and emptied several times before hauling a surface sample on board. As soon as it is aboard, the thermometer is inserted into the bucket. The thermometer should be of a small thermal capacity in order to attain equilibrium rapidly; it should also be checked for calibration errors at a number of points on the scale by comparison with a thermometer of known accuracy, and it should be read with the scale immersed to the height of the mercury column.

When reading the temperature of the condensor intake water either with a fluid thermometer or with an electrical remote-indicating device, the depth of the condensor intake should be noted if the information is going to be used for comparison with the reading of BT-slides.

15.2 NIO-Type in situ salinometer

Based on the original work of Hamon, the National Institute of Oceanography (NIO) in the U.K. some years ago manufactured a comparatively cheap and simple salinity and temperature bridge. (This instrument, slightly modified, is available from Electronic Switchgear in London.) It is briefly described here.

The salinity dependent, electrolytic conductivity of sea water is measured by means of a cell, moulded from mica-loaded epoxy resin, in which carbon composed electrodes are flush fitted within an axial tube through which the water passes. Cleaning is simply carried out by means of a bottle brush and a detergent solution after which the cell should be thoroughly rinsed in distilled water. (In the original NIO instrument the electrodes are made of platinum and are preferably rinsed with hydrochloric acid.)

The measuring probe, connected to 100 m of reinforced cable, contains a thermistor which serves the dual function (1) of automatically compensating for temperature variation errors, when salinity is measured, and (2) as the sensing element for temperature measurements.

The measuring dials are directly calibrated in parts per thousand of sea water salinity and in degrees Centigrade. A hairline cursor and a sensitive null balance indicator enable measurements of salinity, under favourable conditions, to be made to an accuracy of $\pm$0.05‰ S in the range 32-38.2‰ and $\pm$0.1‰ in the range 0.5-32.5‰.

When making a long series of measurements it is desirable to check the bridge occasionally against a known sample of sea water. For field use it is convenient to carry a large thick-walled polyethene bottle with such water. During idle periods between measurements, the probe may be conveniently stored in a bottle of distilled water or clean sea water. Never allow an unclean measuring probe to dry out.

15.3 Water sampling devices (serial)

A water sampler (WS) for collecting at subsurface levels is so designed that it can be closed watertight at any desired depth, and thus the enclosed sample is not contaminated by water at higher levels or lost by leakage after the bottle is brought on board. Because of the great pressures encountered in deep water the WSs are sent down open and then closed at the required depths by means of messengers. To expedite work at sea, water sampling devices are used in series, that is, with more than one bottle on the wire rope so that samples can be taken at a number of depths on the same cast. As it is essential to ascertain the temperatures of the water samples taken at the different depths, the WSs are fitted with frames in which one or more RTs are placed.

The Knudsen WS is still frequently used. It consists of a cylindrical tube with top and bottom plates fitted with rubber gaskets. The plates are connected with a strong spring (inside) through the cylinder. When the instrument is lowered, the water can pass freely through the cylinder.

When struck by a messenger (sliding down the wire rope) the catch is released and the cylinder turns through 180 degrees, thereby pressing the end plates securely against the cylinder and enclosing the water sample. RTs are mounted on the cylinder. This WS holds 1.4 litres.

Recently, the Fishery Board of Sweden has started to use a modern version of the Nansen WS made of polycarbonate. This is also a reversing bottle. It is fitted with two plug valves and holds about 1.7 litres. The two valves, on each end of the cylinder, are operated synchronously by means of a connecting (metallic) rod fastened to the clamp that secures the bottle to the wire rope. When the bottle is lowered, this clamp is at the lower end, and the valves are in the open position so that the water can pass through the bottle. The WS is held in this position by the release mechanism, which passes around the wire rope, but when a messenger, sent down the rope, strikes the release, the WS falls over and turns through 180 degrees, shuts the valves, which are then held closed by a locking device, and reverses

the attached RTs. While reversing the bottle, another messenger that is attached to the wire clamp before lowering is released. This second messenger closes the next, lower WS, releasing a third messenger, and so on (same procedure with Knudsen WSs).

The general procedure for taking water samples and measuring temperatures by means of a serial sampling device (often called a Nansen cast) is as follows. In order to keep the wire rope taut, to reduce the wire angle, a stray weight is attached to the end of the wire rope, usually of 25-50 kg. The depths at which samples are to be collected should be planned before the cast is begun. The number of bottles attached on a single cast is determined by, for example, the strength of the wire (the Fishery Board of Sweden uses a 4-mm stainless steel wire which can tolerate approximately 1 000 kg. In good weather up to twelve WSs are often used). Since the meter wheel is set at zero when the first WS is attached, the entire cast must be lowered by a distance equal to that between the sea surface and the height at which the bottle is put on the wire. After the WSs are lowered to the required depths, they are allowed to remain there for at least five minutes to permit the RTs to reach the surrounding temperature. If the bottles are located at depths of less than about 500 m, their proper functioning may be checked by touching the wire. Just before the messenger is released the wire angle should be estimated or measured. Thus the true depths of the water samplers can be calculated.

15.4 Instruments for determination of currents

15.4.1 Ekman current meter

The essential parts of this instrument (Figure 3) are:

(i) the propeller, the revolutions of which are recorded on a set of dials;

(ii) the compass box, with the device for recording the orientation of the meter;

(iii) the vane which orients the instrument so that the propeller faces the current (not shown in the figure).

The free swing of the instrument is ensured by mounting it on ball bearings, on a vertical axis. The lowering wire is fastened to the upper end of this axis, and a suitable weight is attached below the axis. The instrument is balanced in water so that the axis is vertical. The carefully balanced propeller, with 8 thin, light blades, runs inside a strong protective ring. Inside the protective ring is a lever that can be operated by messengers. With the lever in its lowest position the propeller is arrested, and in this state the instrument is lowered. When the desired depth is reached a messenger weight is dropped, pushing the lever up to its middle position and releasing the propeller. After about five minutes a second messenger weight is dropped which pushes the lever up to the highest position and stops the propeller. The speed of the current is obtained from the reading on the dials, which have to be calibrated against known speeds.

The direction of the current is obtained via a rather elaborate mechanism. A tube extends from above the dial box to a disc on the axis of a cogwheel which turns once when the propeller makes 100 revolutions. This tube is filled with balls of phosphorbronze about 2 mm in diameter. In the compass box, which can easily be removed from the bar to which it is fastened, a magnet swings freely on a pin that runs on agate. As the propeller rotates, the bronze balls fall onto the top of the compass needle and, depending on the heading of the meter, are guided to one of the 10° direction chambers. The compass box is rigidly connected to the vane of the meter, but the magnet of the compass adjusts itself in the magnetic meridian. In general, several shots fall during one observation. The average direction of the current is obtained by computing the weighted mean according to the distribution of the balls.

The use of a compass has the disadvantage that near a steel vessel a compass needle will be greatly influenced by the ship's magnetism. Therefore a compass should not be used for observing directions from a steel vessel at depths of less than 50 m.

The vessel must be anchored during work with this type of current meter. Two anchors are of course still better.

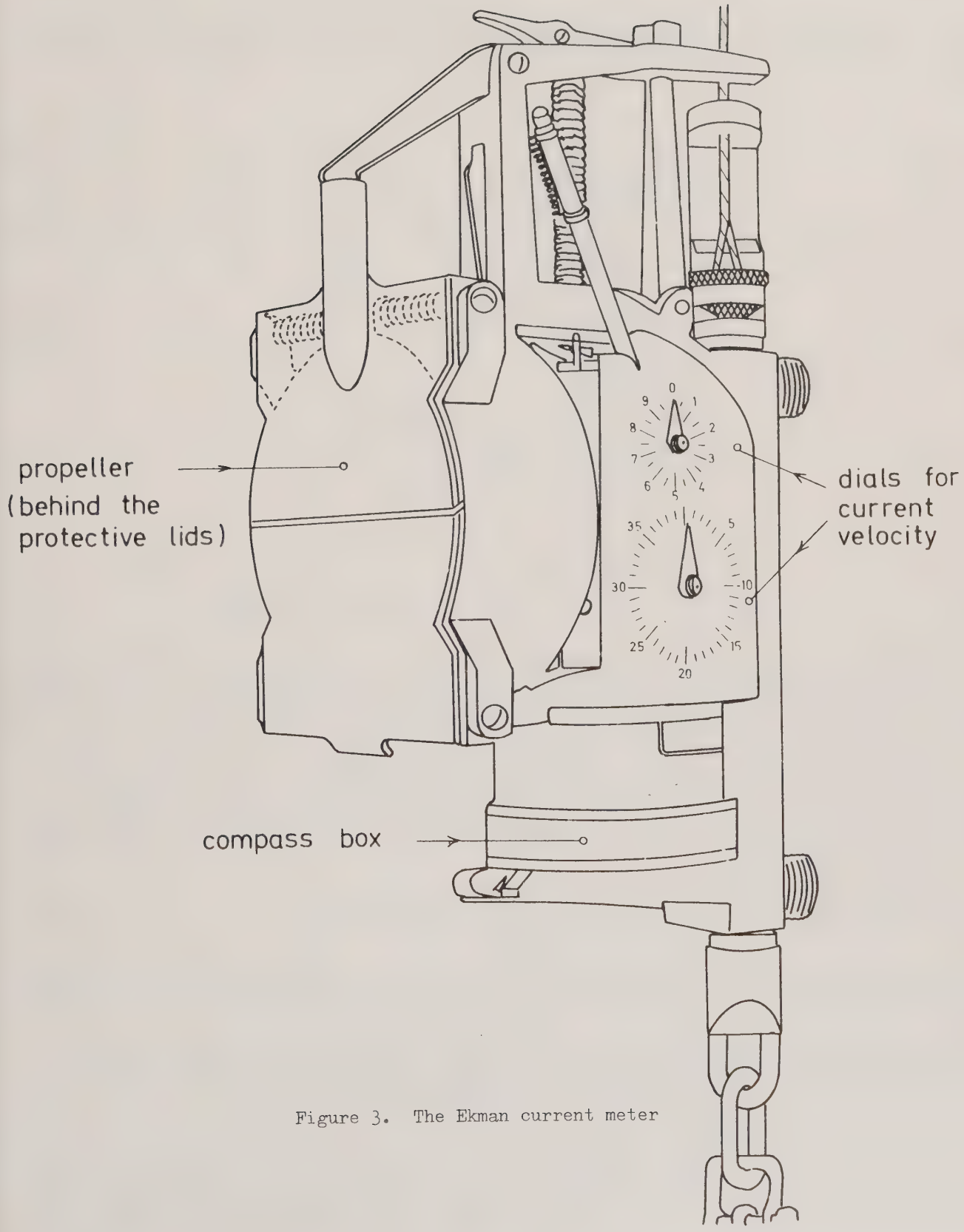

Figure 3. The Ekman current meter

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15.4.2 Carruthers' leaning tube current indicator

A very simple method of determining currents at various depths by means of a modified Carruthers' leaning tube current indicator has been described by Haamer (1974) (see Figures 4 and 5).

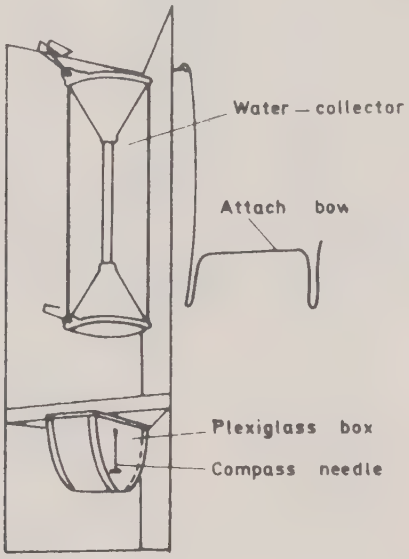

Figure 4. Gelatin pendulum with a water collector

The current meter is based on the pendulum principle, that is, the measuring body consists of a one-point suspended fin, the edge of which is directed against the current. The fin is inclined at different angles to the vertical depending on the speed of the current. Mounted on the fin is a registration device consisting of a plexiglass box filled with a gelatin solution. A compass needle is suspended in the box which shows the angle of the fin in relation to the vertical, as well as the direction of the fin in relation to the north pole of the compass. Before the measurements are started the box is heated and the gelatin melts, so the compass needle hangs freely. When the meter is lowered into the water the gelatin solidifies and thereby locks the compass needle in a position dependent on the strength and direction of the current.

15.5 Instruments for the determination of optical properties

15.5.1 Beam transmittance meter (BTM)

The BTM is designed for the purpose of measuring the attenuation coefficient (c), defined as the absorption coefficient (a) plus the total scattering coefficient (b).

By absorption is meant the sum of the absorption of water (a_w), the absorption of particles (a_p) and of dissolved substances, e.g., yellow substances (Gelbstoff) (a_y). The scattering components are the components of water (b_w) and particles (b_p).

$$c = a_w + a_p + a_y + b_w + b_p = c_w + c_p + a_y$$

The transmittance T, is determined from $T = \frac{F_t}{F_o}$, where F_t is the transmitted and F_o is the incident radiant flux. $c = \frac{1}{r} \ln \frac{F_o}{F_t}$, where r is the path length in metres.

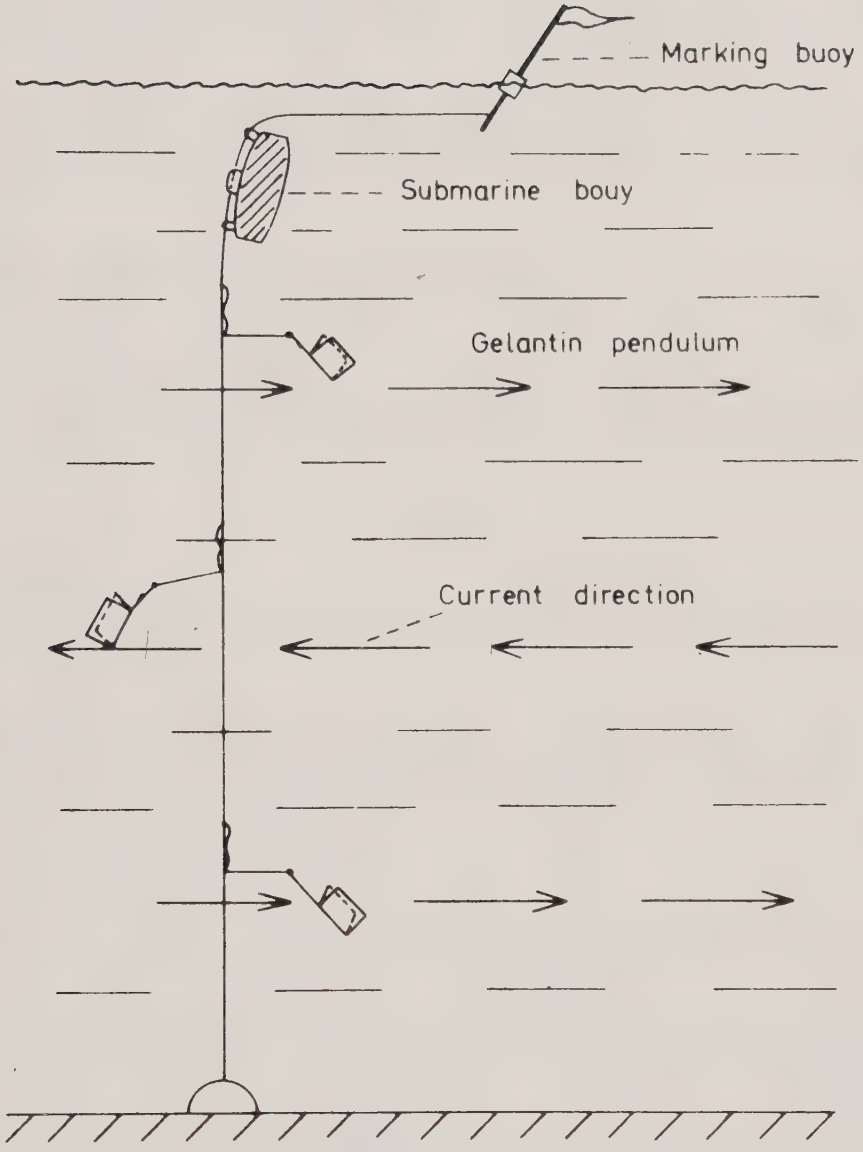

Figure 5. A gelantin pendulum measurement system

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The principle of the BTM is to produce a parallel beam of light which, after passing a water path of fixed length, impinges on a detector, usually a photovoltaic cell. The in situ BTM has for a long time been an effective means of studying the artificial properties especially of coastal waters. Such a meter should be highly efficient in excluding external scattered light.

The BTM is lowered into the water with its light beam horizontal. The lamp is supplied with electricity from the deck through a cable. This same cable connects the photovoltaic cell with a galvanometer. A colour filter is placed in front of the photovoltaic cell.

Performance of measurements: Measurements are made at two wave-lengths, namely, 655 nm (filter RG1, Schott und Gen.) and 380 nm (filters UG 1 + BG 12). The lamp is supplied with an electrical current of 2.0 amp, which must be kept as constant as possible. First readings are taken in air, with the instrument in a dark room (u_a, whereas readings in water are designated u_w).

To get F_o out of the readings in air, the value u_a has to be multiplied with wave-length dependent constants (k_{655} and k_{380}), which are individual for each instrument.

$$c = \frac{1}{r} \ln \frac{k_a . u_a}{u_w}$$

Jerlov (1968) presents a formula to compute the absorption coefficient of the yellow substance at 380 nm:

$$a_y = (c - c_w)_{380} - K(c - c_w)_{655}$$

The values $c_w = 45.10^{-3}$ m^{-1} at 380 nm and $c_w = 304.10^{-3}$ m^{-1} at 655 nm are taken from Jerlov (1968). The factor K, which is the same for a given water mass, is dependent on the average particle size.

This approach assumes that the selective absorbance by particles is proportional to the particle attenuance in the red; it is taken for granted that the absorption of the yellow substance vanishes in the red. The factor K is dependent on the average particle characteristics and is found to be constant for a given water mass.

15.5.2 Measurements of submarine daylight (irradiance)

The simplest way of determining the penetration of submarine daylight is to use a Secchi disc. It is a rough method but accurate enough for practical purposes.

A more accurate method is to use a photovoltaic cell with colour filters and a collector, usually an opal glass. If the irradiance is not too strong, the photoelectric current is proportional to the light energy absorbed. For stronger irradiance a special reducing filter, a sieve, may be used.

To determine the irradiance at 3 wave-lengths measurements are made in turn, with the following filters (Schott und Gen.) : BG 12 + UG 1, UG 9, RG 5 and RG 8. The determinations are then obtained for three wave-lengths: (1) 375 nm, ultra-violet, with BG 12 + UG 1; (2) 535 nm, green, with VG 9, and (3) 680 nm, red, by means of the differences in reading between RG 5 and RG 8. These wave-lengths only apply to the sea surface: if, for example, the measurements are made with a red filter, then as the depth increases, more and more of the yellow part of the spectrum is measured because the light at the other end of the spectrum disappears faster. For green light, however, which in waters off Sweden is usually the wave-length of maximum energy this effect disappears. Measurements are therefore extended below 10 m only with VG 9.

During all the lowerings, the air-values of irradiance are checked by means of a separate photovoltaic cell. If these values change, corrections proportional to the changes must be applied.

The measurements are preferably carried out around noon. In any case there is a dependence on the sun's altitude (Joseph, 1952).

Transferance into total energy values is a little complicated. It can be studied in the paper by Jerlov (1951). A simpler way is to carry out measurements with a so-called quanta meter.

There is a rough relation between Secchi disc depth, D m, and the attenuation of daylight C_d (per metre) in green or blue, if D is not too large. Poole and Atkins (1929) presented the formula

$$D \times C_d = 1.7$$

Instead of C_d the transmittance T (pr m) or the depth Z_1 and Z_{10}, down to which only 1% and 10% respectively of the surface irradiance (in blue-green) reaches (Table I).

The attenuation coefficient c_d of a daylight measurement differs however from c_B computed from the measurements with a beam transmittance meter. Jerlov (1951) discusses this problem and shows that the influence of scattering is much less on c_d than on c_B.

Table I

T % pr m	z_{10} m	z_1 m	c	D (for T green or blue)
10	1.0	2.0	2.30	
15	1.2	2.4	1.92	
20	1.4	2.8	1.65	
25	1.7	3.3	1.40	
30	1.9	3.8	1.21	
35	2.2	4.4	1.05	
40	2.5	5.0	0.92	
45	2.9	5.8	0.79	
50	3.3	6.6	0.70	2.4
55	3.9	7.7	0.60	2.8
60	4.5	9.0	0.51	3.3
65	5.4	10.7	0.43	4.0
70	6.5	12.9	0.36	4.7
75	8.0	16.0	0.29	5.9
80	10.6	21.2	0.22	7.7
85	14.2	28.4	0.162	10.5
90	21.8	42.8	0.108	15.8
95	44.9	89.8	0.0514	?

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15.6 References

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This chapter was prepared by A. Svansson.

Information has been taken from Sverdrup et al., 1970 (15.1.1, 15.3, 15.4.1) and from Unesco/IOC, 1972 (15.1.2).

16. METHODS FOR THE DETERMINATION OF PHYSICAL AND INORGANIC PARAMETERS IN WATER

16.1 Determination of sea water salinity

16.1.1 Titrimetric determination of chlorinity, calculation of salinity and density

16.1.1.1 Introduction

The titrimetric method for determination of chlorinity of sea water is capable of giving very accurate and precise results. However, to achieve these high quality results, and for the intercomparability between different laboratories, it is absolutely necessary to follow the given procedure to its smallest detail.

The halogen compounds in sea water (chlorides, bromides and iodides - here termed chlorides) may be precipitated as very slightly soluble silver complexes - silver chloride - by addition of a soluble silver salt. In the titration, a solution of silver nitrate ($AgNO_3$) is added from a calibrated burette to a carefully measured volume of the sea water sample. The silver nitrate solution has to be standardized daily by titration with standard sea water (SSW).

When all chlorides are precipitated in the sample, free ions of silver from the added solution are detected with an indicator, which changes its colour. Thus the addition of silver nitrate solution can be stopped at the proper moment and the exact amount of solution that has been consumed can be measured and recorded.

Extreme cleanliness is essential. It is not enough that the pipette and the burette look clean. The most important point is that no drops or streaks of liquid be allowed to adhere to the glass surfaces. Several liquids can be used for cleaning, dichromate-sulphuric acid or alcoholic sodium hydroxide. Synthetic detergents can also give a good result. They are less harmful to the glass surfaces than sodium hydroxide. Beakers, glass rods and other equipment must be washed in sulphuric acid occasionally.

At best, the titration should take place in a dust-free room with constant temperature, preferably 20°C. The analyst must strive to eliminate the different sources of error as far as possible. It should not be assumed that a sample is impaired by deficiencies at the outset and that therefore only a low quality analysis need be made.

16.1.1.2 Reagents and auxiliary chemicals

Silver nitrate solution. 37.11 g $AgNO_3$ is dissolved in distilled water and diluted to 1 000 ml. (For routine work a greater volume is normally prepared.) The normality of the solution is chosen so that the burette reading in dml is approximately the same as the chlorinity of the sample. This corresponds to a normality of about 0.22. When using this solution an alpha-value greater than 0.10 (see below) may be obtained. In this case an additional volume of distilled water has to be added to the solution. The size of this volume depends on the pipette used in the analysis. The volume of distilled water is determined by titrating SSW (see 16.1.1.5), whereby a certain alpha-value is obtained. This is multiplied by 1 000 and the number of litres of solution prepared, and finally divided by the burette reading in dml. The result is the volume of distilled water in ml.

On the other hand it may happen that the solution is too weak and an alpha-value less than -0.10 is obtained. If so, a certain weight of silver nitrate has to be added: the alpha-value (from titration of SSW) is multiplied by 37.11 and the number of litres of solution prepared, and divided by the burette reading in dml. This number of grammes of silver nitrate must be weighed on an analytical balance.

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Indicator, sodium fluorescein and starch. 100 mg sodium fluorescein is dissolved in 1 000 ml distilled water. 2 g starch (soluble) is dissolved in 100 ml distilled water by heating. This solution cannot be stored for more than 4-5 days. Equal volumes of the two solutions are mixed in a 100 ml pipette bottle.

Dichromate-sulphuric acid. This can either be bought from the suppliers of chemicals or be prepared by carefully dissolving about 5-10 g potassium dichromate ($K_2Cr_2O_7$) in 250 ml concentrated sulphuric acid (H_2SO_4). This mixture is used for cleaning the glassware.

Alcoholic sodium hydroxide solution. 20 g of sodium hydroxide (NaOH) is carefully dissolved in 20 ml distilled water and alcohol (95%) is added up to 100 ml. This cleaning solution etches glass and may therefore not be used for more than ten minutes.

Stopcock lubricant, of silicon type. The grounded stopcocks in the pipettes and burettes are slightly lubricated. A better, but more expensive alternative is to use stopcocks of P.T.F.E. (Teflon) because they need no lubrication and they do not get stuck.

Note: The cleaning solutions are very dangerous to prepare and handle. They should by no means be handled by untrained staff or other persons who are unfamiliar with the risks. If swallowed the solutions are fatal. They should never be allowed to come into contact with eyes, skin or clothes.

16.1.1.3 Equipment

- Burette with automatic zeroing as shown in Figure 1. This is for ocean water (10-20‰ Cl). For waters from estuaries, where the salinity is low, a burette like that in Figure 2 is used. This has two graduated zones, above and below the bulb. The graduation should be in double millilitres (dml) and 2/100 thereof. The graduation lines should extend around two thirds of the periphery of the burette. The burette (as well as the pipette below) can either be specially ordered from a glassworker's shop or obtained from various manufacturers of oceanographic equipment (for instance, Hydro-Bios, Kiel, Federal Republic of Germany). Alternatively, an ordinary burette of sufficient volume may be used, in which case, the graduation has to be read to every second ml only, as one dml corresponds to two ml. Whichever burette is used, it should be calibrated.

- Pipette: a so-called automatic pipette or Knudsen pipette (Figure 3). The glass should be of Geräte-quality or equivalent, and be free from air bubbles and irregularities. The volume, which has to be checked, should be close to 15 ml at 20°C.

- Bottle with grounded siphon (Figure 4). The bottle should be made from brown glass and is used for storage of the silver nitrate solution.

- Bottle with water-lock (Figure 5), for storage of the standard sea water (SSW).

- Hoses of plastic, with an inner diameter of 7-8 mm.

- Glass tubes, with an outer diameter of 8-9 mm.

- Spray bottles, as in Figure 6, and of 500 ml volume.

- Beakers, of Pyrex glass or equivalent. 250 ml volume, height 90 mm, diameter 65 mm. These are used for the titration of the samples. 4-5 beakers are required.

- Glass rod, for manual stirring of the samples. Length 180 mm, diameter 5 mm.

- Bottles, for chemicals and rinsing water. Volume 250 ml with grounded stoppers. 3-4 are required.

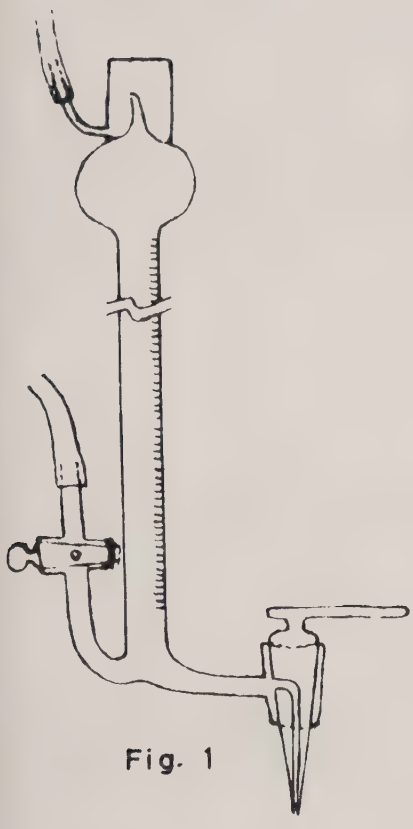

Fig. 1

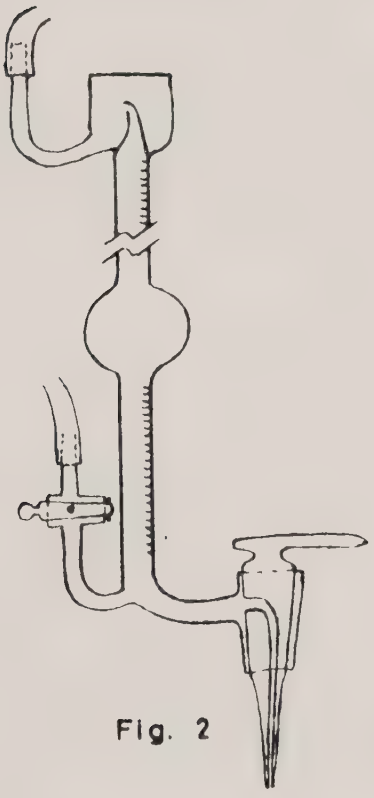

Fig. 2

Fig. 3

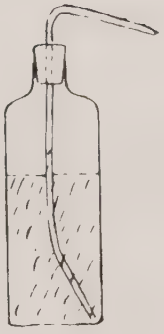

Fig. 6

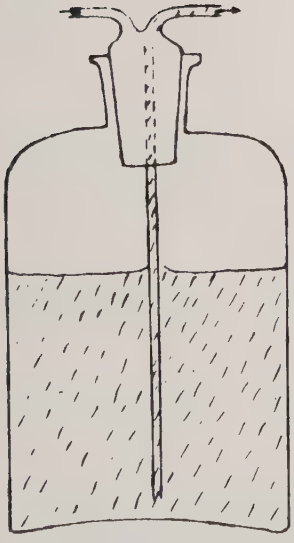

Fig. 4

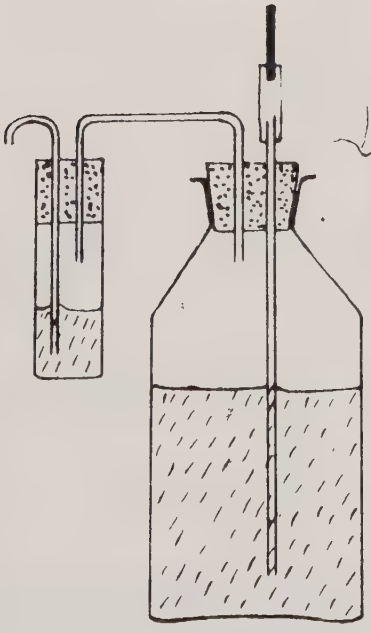

Fig. 5

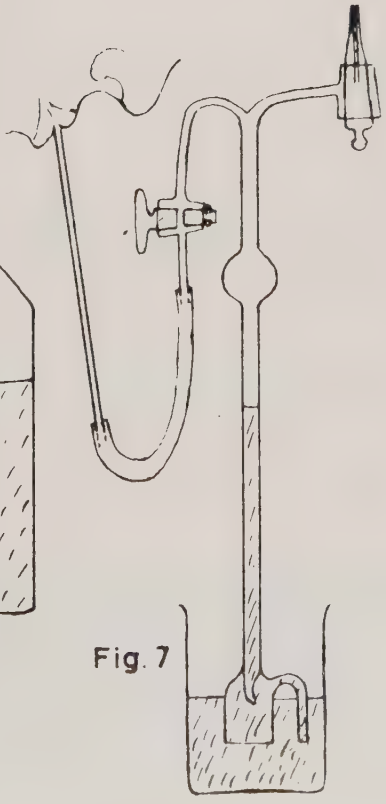

Fig. 7

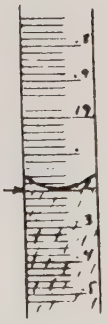

Fig. 8

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- Pipette bottle, capacity 100 ml. The indicator is stored in this bottle. The pipette should deliver a volume of 2 ml.

- Filter paper, sheets of about 40 x 40 cm.

- For continuous routine work with chlorinity titrations, it is advisable to buy an automatic magnetic stirrer with speed control. Preferably the motor is mounted under the table top, so the beaker can be placed directly on the table while the titration is performed. The stirring magnets, one of which must be in the sample during the titration, could be of 35-40 mm in length and 7-8 mm in diameter.

- Forceps, for handling the stirring magnets.

- Watch-glass, for storing the stirring magnets while they are not in use.

16.1.1.4 Preparation of the equipment

All glassware has to be cleaned before use. The cleaning is preferably done with a synthetic detergent and distilled water or with the dichromate-sulphuric acid. The alcoholic sodium hydroxide is used only if the first treatment is ineffective. The best result is obtained if the solutions are hot (about 90°C).

The cleaning solutions - except the detergent - are very dangerous to handle and use. They are highly corrosive and can cause serious harm to human beings.

Using a rubber pipette, Peleus ball or similar aid, the solution is sucked into the pipette and then pumped back and forth for five minutes. After this the pipette is rinsed with distilled water at least three or four times. Only the detergent solution may be sucked by the mouth.

The burette is rinsed with water a few times, so no silver nitrate solution remains. After this the burette is turned upside-down and the normal upper end is introduced into the hot cleaning solution (Figure 7). To the inlet pipe a hose is connected and with this the solution is sucked up. The solution is pumped back and forth for at least five minutes, just as for the pipette. Alternatively, the cleaning solution can be left standing in the burette for a while longer. The alcoholic sodium hydroxide should by no means be left in the burette for more than ten minutes.

When the instruments are clean the stopcocks are removed and they - as well as their grounded sites - are washed similarly. The stopcocks may be lubricated, but only very slightly. Lubricant should never be allowed to escape into the liquid channels; if this happens, the cleaning procedure has to be repeated.

The cleaning must be complete. Neither dirt, drops or streaks of water may be left on the glass surfaces. This is easily checked by letting the instruments rest for a while and ensuring that no new drops have appeared at the outlets. When cleaning the pipette care must be taken that no water is left in the air-inlet pipe.

Glass rods, beakers, stirring magnets, etc., must be cleaned in dichromate-sulphuric acid now and then.

When the burette is absolutely clean it must be calibrated before use. To the lower inlet tube is connected a hose from a bottle of distilled water. The temperature of the room and the water should be as close to 20°C as possible. The burette is filled with distilled water; 2.5 dml is drained into an absolutely dry beaker or flask, which has been pre-weighed. The weight of the 2.5 dml is determined with an analytical balance. The burette is filled again and 5.0 dml are drained and weighed in the same manner. The procedure is continued for every 2.5 dml through the entire volume of the burette. The calibration is repeated two more times. The temperature of the water is measured at every weighing and the volume

corrected to 20°C. If the burette correction is 0.00, the weight differences agree with the volume differences. If not, a correction term has to be applied. This is so assigned that a positive correction (e.g., +0.02) should be added to the burette reading.

Now the burette is connected to the silver nitrate system. The connexions are made from glass tubes and plastic hoses. Start connecting the right-hand outlet of the siphon (see Figure 4) to the lower inlet tube of the burette. Then blow in the hose at the left-hand outlet from the siphon. This starts the circulation and the burette is filled. Finally, connect this hose to the upper outlet pipe of the burette so the excess of silver nitrate solution is sucked back to the supply bottle.

16.1.1.5 Procedure

Before starting the work:

- check the burette reading for control of possible leakage;
- drain three burette volumes of silver nitrate solution into a clean beaker; store this solution in a dark glass bottle with grounded stopper; it may be re-used when new silver nitrate solution is prepared;
- rinse titration beakers, glass rods and their storage beaker, stirring magnets and watch-glass with distilled water.

Titration of standard sea water (SSW):

This titration is made for calibration of the silver nitrate solution. The same Knudsen pipette is used for measurement of SSW and sea water samples and therefore its volume does not have to be carefully calibrated regularly. However, the pipette's volume must be close to 15 ml at 20°C in order to keep the alpha-values within the limits of the Knudsen Hydrographical Tables (Knudsen, 1901).

The titration is performed every day before starting the analyses and also every time new silver nitrate solution is added to the supply bottle.

Before the titration, the pipette is carefully rinsed with water of about 35‰ salinity. The SSW is stored in a glass bottle with water-lock (Figure 5) so its concentration is not changed by evaporation.

It is very important that the calibration titration is made in exactly the same manner as the analyses. The titration is made on at least three samples and the results may not differ more than ±0.01 dml. The arithmetical mean is used in the calculations.

(The SSW is obtained from: IAPSO Standard Sea Water Service, Charlottenlund Slot, DK-2920 Charlottenlund, Denmark.)

Titration of sea water samples:

(1) Place the stirring magnet in the sample beaker;

(2) Record the number of the sample bottle on the log sheet, shake the bottle and open it;

(3) Fill the pipette to half with sample, shake and drain out; this is done twice. Water should never be allowed into the bent air tube of the pipette. This leads to errors in the sample measurement, and cross contamination;

(4) Fill the pipette completely and close it by turning the stop-cock 90 degrees;

(5) Close the sample bottle;

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(6) Wipe the exterior of the pipette with a piece of filter paper without touching the outlet opening; hang the pipette vertically;

(7) Turn the stop-cock another 90 degrees and let the sample flow out from the hanging pipette into the sample beaker;

(8) Add 2 ml of indicator;

(9) The last drop of the sample is forced out of the pipette by blowing in the air tube;

(10) Drain a few drops of silver nitrate solution out of the burette into a collecting beaker; wipe the outflow tip of the burette with a filter paper and fill the burette so some drops of solution overflow;

(11) Place the sample beaker on the magnetic stirrer, start the motor and open the outflow of the burette. When the sample turns slightly pink, the outflow is stopped again. Sometimes the precipitate in the sample is flocky rather than finely divided. When this happens the stirring is stopped and the sample is manually stirred, vigorously, with a glass rod, so the flock is disintegrated. If another drop of the sample has appeared at the outlet of the pipette, this water is added by blowing once again. Place the beaker below the burette and start the magnetic stirring. Silver nitrate solution is added at the end of the titration in very small drops, by means of the glass rod. The titration is complete when a faint, pink hue persists; even on stirring for some minutes, the colour should not change;

(12) Take the burette reading and record this on the log sheet. The reading is made at the lower meniscus of the liquid (Figure 8). If there is any doubt about the end point of the titration (the change in colour) the reading is taken and then after a small addition of silver nitrate solution, another one is made. From the appearance of the sample it is decided which reading to use. If there still is any doubt, a new portion of the sample is titrated;

(13) Rinse the stirring magnet and glass rod over the beaker with the titrated sample. Discard the content into a bucket and/rinse the sample beaker carefully.

All silver chloride can be saved and later refined to new silver nitrate. This cuts the costs of the analyses in the long run.

After the work is finished:

(14) Fill the burette completely;

(15) Rinse the beakers, glass rods, stirring magnets, etc., with distilled water; turn the beakers upside down;

(16) Rinse the pipette with distilled water and let it remain filled;

(17) For security, the hose to the burette inlet should be closed with a hose clamp;

(18) A small beaker should be placed below the burette outlet to collect possible spill.

16.1.1.6 Calculation of chlorinity, salinity and density

Chlorinity and salinity: The alpha-value is the numerical difference between the chlorinity of the SSW (given on the ampoule) and the burette reading in dml when titrating the SSW sample. This value is calculated and recorded on top of the log sheet; but before doing this, the burette reading must be corrected with respect to the possible error in the graduation./

Salin 7

DETERMINATION OF SALINITY

Analyst: .S.A..Linity..... Sampling period: .1974-05-12--29.. Sample box nr.: ..93...

Burette No. 21		Pipette No. 9		alpha-value -0.015		Date of titration 1974-06-03			
Sampling station nr	depth m	Sample bottle nr	Burette reading dml	Burette correction dml	$AgNO_3$ dml	k	Cl ‰	S ‰	Remarks
F72	0	3316	3.57	0.02	3.59	+0.07	3.66	6.64	σ_o = 5.30
	5	3317							
	10	3318							
	15	3319							
	20	3320							

Figure 9. Example of a log sheet for salinity titration

In the Knudsen Hydrographical Tables[1/], pages 23-34 cover the range of alpha -0.150 to +0.150. The calculated alpha-value and the corrected burette reading in dml are entered in the table. It should be remembered that if an ordinary burette was used the reading must be divided by two in order to obtain dml (double millilitres) as this unit is the one used in the Knudsen Tables. If the alpha = -0.015 and the burette reading is 3.59 dml, the k-term (page 28) is +0.07. This k-term is added to the burette reading to give the chlorinity: 3.59 + 0.07 = 3.66‰.

On pages 1-22 the salinities are found. Page 3 shows that the chlorinity 3.66‰ corresponds to a salinity of 6.64‰ (brackish water) and σ_o, which is described below, is 5.30.

1/ May be obtained from Hydro-Bios, Kiel, Federal Republic of Germany

Salin 8

There is also a more simple way to calculate the salinity. For this, "Kalle's Circular Slide Rule for Salinity Determination" (Kalle, 1955) is used. This determines the salinity directly from that amount of silver nitrate which was consumed in the titration.

The slide rule consists of two plastic sheets between two pieces of perspex. On one sheet is printed the consumed volume of silver nitrate solution in dml and on the other the salinity in grammes per kilogramme. The two plastic films are turned so that the volume (in dml) consumed in the titration of the SSW corresponds to the salinity (not chlorinity) of the SSW. The positions of the films are now fixed with adhesive tape. The salinities corresponding to the burette readings can now be read on the scales (do not forget the burette correction term). The results from the slide rule are easier to obtain than those from the Knudsen Tables and the greatest error is 0.02‰.

Density: The density of water depends on salinity, temperature and hydrostatic pressure. In areas with water depths of less than a few hundred metres, the pressure may be neglected. The density is often assigned ρ and is always greater than and close to 1. Thus it must be accurately determined to several decimal places. To simplify the treatment of the density figures, the expression used is $\sigma = (\rho - 1) \times 1\,000$. If $\rho = 1.00844$ then $\sigma = 8.44$.

The $\sigma_o = 5.30$, which was found in the example above from the Knudsen Tables, represents the density at 0°C. On pages 39-42 of the Tables the density at any temperature can be found from the temperature and σ_o. In the table is found a term D, which has to be subtracted from σ_o to obtain σ_t; $\sigma_t = \sigma_o - D$. If in the example the $\sigma_t = \sigma_{20}$ is wanted from $\sigma_o = 5.30$ we have to interpolate between 1.96 and 2.03. The proper D value is thus 1.98, which is subtracted from 5.30. The σ_{20} is then 3.32 and the density $\rho = 1.00332$.

In order to avoid all interpolation, Publications de Circonstance, no. 11 (Knudsen, 1904) can be used, because it is tabulated in more detail.

The simplest way for density calculations is to use a nomogram called "Kalle's Fluchtentafeln zur Bestimmung der Dichte aus Salzgehalt und Temperatur des Meerwassers". Here σ_t is obtained from salinity S, and temperature T (σ_o has not to be recorded). The actual values on the S and T axis are connected with a ruler or a thin string. Where this intersects the σ_t -lines (four possible, choose the one which belongs to the T-axis used) the σ_t is read.

16.1.2 Hydrometric determination of salinity

16.1.2.1 Introduction

A crude method for determining salinity in sea water is to use a hydrometer. Among others, there are specific gravity (density) hydrometers and salinity hydrometers. Each must have its data reduced to a temperature for which it was calibrated (e.g., 17.5°C).

The temperatures of the sample and of the surrounding atmosphere should be nearly equal during the observation; otherwise the temperature of the sample will be changing, causing not only differences in density but also doubt as to the actual temperature. If an observation is made at some other temperature than that for which the hydrometer is designed the reading will be erroneous. The magnitude of the error will depend upon the thermal expansion of the hydrometer.

16.1.2.2 Procedure

The hydrometer should be clean, dry, and at the temperature of the sample before immersing to make a reading. In order to remove dirt and grease it may be necessary to wipe the instrument with a cloth soaked with acetone or alcohol. After this the hydrometer should not be touched by hands directly, but rather with a dry cloth.

The sample in which the observation is made should be contained in a clear, smooth glass vessel of suitable size and shape. The hydrometer should be slowly immersed in the liquid slightly beyond the point where it floats naturally and then allowed to float freely. The scale reading should not be made until the sample and hydrometer are free from bubbles and at rest.

Salin 10

16.1.3.2 Procedure

(1) When the measurements start, the cell of the salinometer contains distilled water. This is drained and a portion of old (that means older than two days) SSW is sucked into the cell. This water is stirred for some seconds and then drained. The cell is thus flushed. Another portion of the old SSW is introduced. Make sure that the cell is entirely filled so that water can be seen at the overflow. If air bubbles are trapped in the cell no stable readings can be obtained.

(2) This second portion of the old SSW is stirred and the temperature is measured by depressing the TEMPERATURE/SALINITY switch to the temperature position. After this the CONDUCTIVITY RATIO dials are turned until the meter is nulled. This conductivity ratio usually does not differ much from that of a fresh SSW. Thus it serves as a day-to-day control of the entire measuring system. Finally the sample is drained.

(3) The procedure is repeated with fresh SSW. Rinse with one portion and fill up with another one. On the SSW ampoule is printed the actual chlorinity of the SSW. If this is 19.375‰, it can be seen in the conductivity ratio table on the salinometer that the corresponding salinity is 35.0019‰ and the conductivity ratio is 1.00004. While the sample is continually stirred, the CONDUCTIVITY RATIO dials are turned until they show 1.00004. The NULL/TEMPERATURE INDICATOR meter is probably not in null position, but this is obtained by turning the STANDARDIZE dials. When the null position is reached the instrument is standardized. The STANDARDIZE dials should not be turned until the standardization process has to be repeated. In the record book for the instrument, the reading of the STANDARDIZE dials is written together with the measurement temperature.

(4) Drain the SSW and rinse with sub-standard. Introduce another portion of the sub-standard. The temperature is checked while stirring. It should be as close as possible to that of the SSW. Now determine the conductivity ratio for this sub-standard. The result is recorded in the book too.

Now this sub-standard is used for periodical check-up of the standardization during the working day. A check-up after each wooden case of samples (50-56 samples) has proved to be a good habit. The recordings in the book are very important as a control of the long-term behaviour of the measuring system.

(5) After standardization the samples are measured in the same way. A first portion is stirred and drained to rinse the cell and a second portion is measured. The conductivity ratio and the temperature for each sample is recorded on the log sheet.

That portion of the sample which has been measured in the cell may be drained back into the sample bottle. Thus enough water is saved for checking up salinities which seem to be dubious.

16.1.3.3 Calculation of the actual salinity

To compute salinity from the conductivity ratio measured at a temperature other than $20^{\circ}C$, the temperature of the water sample also must be determined. From Table IIb in the International Oceanographic Tables, determine the correction term using the observed ratio and temperature. Add the correction (Δ_{20}) to the observed ratio (R_t) to give the ratio at $20^{\circ}C$ (R_{20}).

Example: Observed ratio is 0.86541 at $24^{\circ}C$

$$R_t = 0.86541$$
$$\text{from Table IIb} = \underline{\quad -24}$$
$$R_{20} = R_t + \Delta_{20} = 0.86517$$

From Table IIa, the salinity = 29.763‰ (the last digit has to be found by interpolation)

pH 1

Station code	Depth (metres)	Sample number	R_t	Δ_{20}	R_{20}	Salinity ‰
P3	0	2216	0.86541	-24	0.86517	29.763
	5	2217				
	10	2218				
	15	2219				

Figure 10. Example of a log sheet

16.2 Determination of pH

16.2.1 Introduction

The determination of pH is performed electrometrically, using a glass electrode, with a calomel electrode as reference.

For the determination of pH with the highest precision and accuracy, which represents normal practice in oceanography, the full details of this method should be followed. If the demands are less stringent (precision and accuracy of $\pm$0.1 pH unit or less) a simplified procedure, omitting the closed measuring cell and the constant temperature bath, may be applied. The calibration procedure is generally applicable, however.

16.2.2 Sampling

It is important to minimize interaction between the sample and the carbon dioxide in the air. Hence the same care and precautions as in oxygen sampling are advocated. The sample is drawn via a hose from the water sampler(immediately after the oxygen sample) into a 100 ml glass bottle with a ground stopper. The sample should be analysed within one hour after sampling.

16.2.3 Buffer solutions

As pH values are temperature dependent, the pH values of the following solutions are given in Table I. For a proper calibration at least two of the solutions are required. The choice of solutions is governed by the pH range of the samples.

Bates 1:1 buffer. Potassium dihydrogen phosphate, KH_2PO_4; 3.39 g and disodium hydrogen phosphate, Na_2HPO_4, 3.53 g are dissolved in distilled water free of carbonates and diluted to 1 000 ml.

Bates 1:3.5 buffer. Potassium dihydrogen phosphate, KH_2PO_4; 1.179 g and disodium hydrogen phosphate, Na_2HPO_4, 4.30 g are dissolved in distilled water free of carbonates and diluted to 1 000 ml.

pH 2

Palitzsch borax buffer. Sodium tetraborate, $Na_2B_4O_7.10H_2O$, 9.554 g, boric acid, H_3BO_3, 6.202 g, and sodium chloride, NaCl, 1.462 g are dissolved in distilled water free of carbonates and diluted to 1 000 ml.

Bates 0.01 M borax buffer. Sodium tetraborate, $Na_2B_4O_7.10H_2O$, 3.814 g is dissolved in distilled water free of carbonates and diluted to 1 000 ml.

The sodium and potassium salts should be dried before weighing in. It is always advisable to keep a supply of these salts in a dessicator containing silicagel; thus time-consuming drying in an oven is avoided. Some authors recommend recrystallization of borax before use. This should be done from water below 50°C. To prevent partial dehydration of borax, it should be kept over a saturated solution of sugar or sodium chloride or slightly moist sodium bromide, in a closed container. The boric acid could be kept in the same manner.

Distilled water free of carbonates is prepared by boiling distilled water for a while and, preferably, passing nitrogen gas through during the cooling. The water should then immediately be used for the preparation of solutions.

Table I

Standard pH values of the buffer solutions

Temp. °C	Bates 1:1	Bates 1:3.5	Palitzsch borax a/	Bates 0.01 M borax
0	6.984			
5	6.951			
10	6.923	7.472		9.332
15	6.900	7.448		9.275
20	6.881	7.429	8.510	9.225
25	6.865	7.413	8.459	9.180
30		7.400		9.139
35				9.068

a/ For Palitzsch buffer only two values are given because at other temperatures the values are uncertain, as found in ICES intercalibration tests and elsewhere

16.2.4 Equipment

- Water bath, thermostated, with pump for external circulation of the thermostated water. Should be capable of holding a selected temperature to within ± 0.2°C.
- Measuring cell and heat exchanger, of glass, constructed as in Figure 11. These items and connecting tubes should be as small as possible in order to reach thermal equilibrium quickly, and should not require too much sample water for washing and measurement. The electrodes should be kept in sample water between measurements. Only during longer storage should distilled water be used.

pH 3

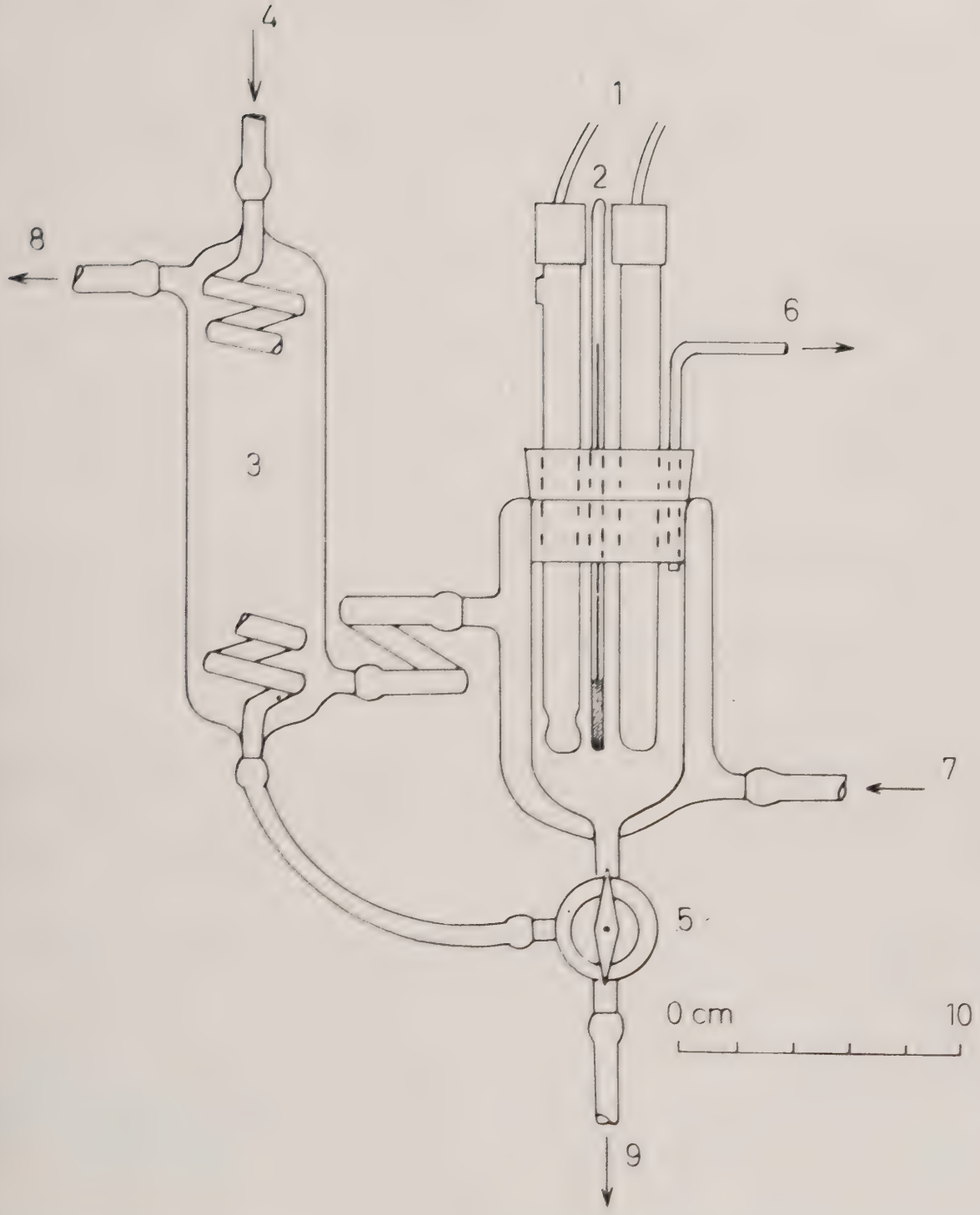

Figure 11. Thermostated pH-cell and heat-exchanger

(1) electrodes
(2) thermometer
(3) heat exchanger (narrow bore)
(4) sample inlet
(5) teflon valve
(6) sample overflow
(7) thermostated water inlet
(8) thermostated water outlet
(9) to waste

pH 4

- Electrodes, any commercial type of good quality may be used. (W. Ingold AG, Zürich, Switzerland, has a glass electrode called LOT 201, which may be a good choice because it has a fast response and works properly even at low temperatures.)

- pH meter of good quality. An instrument with a galvanometer should be provided with scale expansion facilities. For shipboard use an instrument with digital display is preferred, as the galvanometer needle is influenced by the movements of the ship.

16.2.5 Calibration

For the exact handling of the pH meter, the instruction manual supplied with the instrument should be consulted.

The pH meter is standardized with Bates 1:1 phosphate buffer at the chosen constant temperature (20 or 25°C). Because most electrode pairs are not entirely linear over the entire pH scale, the slope (mV/pH) of the electrodes should be corrected for. Immerse the electrodes in Bates 0.01 M borax buffer (rinse well). If a slope correction is provided on the instrument, this is used to get the proper pH reading according to the values in Table I. (In this case the temperature compensation is set at the measuring temperature and not shifted after calibration of the instrument.) If no slope correction is available, the temperature compensator can be used because it works similarly. As a final check Bates 1:3.5 buffer can be recommended.

Instead of Bates 0.01 M borax buffer, Palitzsch borax buffer can be used. The advantage of this is that the pH value at room temperature is close to the maximum pH value of sea water.

Rinse the measuring cell with sea water several times to remove all traces of buffer solutions. Keep the cell filled up with sea water.

16.2.6 Measurement

The sample is brought through the heat exchanger into the cell with the aid of a siphon. Rinse the cell three to four times with the sample. Fill the cell completely and let the sample reach constant temperature. (A small thermometer should extend into the cell as in Figure 11.) For high precision and accuracy, the temperature difference between calibration and measurement should not exceed $\pm 0.5^{\circ}C$. Make a note of the pH and the measurement temperature, and pass out the sample.

From the writer's experience no problem is encountered when passing from a sample with a high pH to a sample with a low pH (as is the case at the halocline). However, after measurement of a sample with a low pH, additional rinsing may sometimes be required.

16.2.7 Calculation

The measured pH value is not the true pH value (*in situ* value) of the sampled water. The measured - or apparent - value has to be corrected for the temperature difference between measurement conditions and those at the sample depth in the water. If the sample was obtained from about 500 m or deeper, a further correction for the difference in hydrostatic pressure must be applied.

The formula for calculation of the $pH_{\underline{in\ situ}}$ is then:

$$pH_{\underline{in\ situ}} = pH_{measured} + 0.0118(t_2 - t_1)$$

where: t_1 = temperature *in situ*

t_2 = measurement temperature

This formula is valid for work in areas with water depths not exceeding 500-1 000 m (depending on the accuracy desired). The complete correction formula is:

$$pH_{\underline{in\ situ}} = pH_{measured} + 0.0118(t_2 - t_1) - \beta \cdot Z$$

The last term $\beta \cdot Z$ is a correction factor for the difference in hydrostatic pressure between the sampling depth and the laboratory at the sea surface. Z is the sampling depth in metres and the β-value is obtained from Table II.

Table II

$pH_{measured}$	β	$pH_{measured}$	β
7.5	35.10^{-6}	8.0	22.10^{-6}
7.6	31	8.1	21
7.7	28	8.2	20
7.8	25	8.3	20
7.9	23	8.4	20

16.3 Determination of turbidity

A brief introduction to the turbidity concept, a description of an instrument and the measurements are given in chapter 5.6.

This "analysis" is a purely instrumental operation. The performance of the simple measurements can be fully understood from the text cited above. Therefore reference is given to the manufacturer's manual for a detailed description of the handling of the chosen instrument.

16.4 Determination of dissolved oxygen and oxygen saturation

16.4.1 Introduction

The method described is the common Winkler method, modified by Carritt and Carpenter. It is applicable to all kinds of natural waters as well as most waste waters. The method is based on the following principle: Manganous ions are precipitated in alkaline medium, forming manganous hydroxide. This is oxidized by the dissolved oxygen: $2Mn(OH)_2 + O_2 \longrightarrow 2MnO(OH)_2$. The manganese hydroxide is dissolved with acid and reduced by iodide ions: $MnO(OH)_2 + 4H_3O^+ + 3I^- \longrightarrow Mn^{2+} + I_3^- + 7H_2O$. The I_3^- ions produced are determined by titration with thiosulphate ions:
$2S_2O_3^{2-} + I_3^- \longrightarrow S_4O_6^{2-} + 3I^-$

16.4.2 Reagents

Manganous sulphate, $MnSO_4.H_2O$. 500 g is dissolved in distilled water and diluted to one litre. Store in a plastic bottle. ($MnSO_4.4H_2O$, 670 g, or $MnCl_2$ in equivalent amount may be used.)

Sodium iodide, NaI. 600 g is dissolved by warming in the smallest possible volume of distilled water.

Sodium hydroxide, NaOH. 320 g is dissolved in the smallest possible volume of distilled water.

O_2 2

Sodium azide, NaN_3. 10 g is dissolved in the smallest possible volume of distilled water. (This reagent is used in order to destroy nitrite in stagnant waters.)

These sodium reagents are mixed together and diluted to one litre. Store in a plastic bottle. These reagents are very concentrated and generally will not become clear until they are diluted to the full volume. If crystals are formed, the clear liquid is decanted into another vessel.

Sulphuric acid, H_2SO_4 (1 + 1) solution. Concentrated acid is mixed with the same volume of distilled water. The acid is slowly poured into the water under constant mixing and cooling.

The reason for using sulphuric acid instead of the commonly recommended concentrated phosphoric acid is because during field work there is a great risk of contaminating samples for the phosphate analysis when phosphoric acid is used.

Sodium thiosulphate, $Na_2S_2O_3.5H_2O$, 0.02 N. 25 g is dissolved in distilled water. 50 ml isobutylalcohol is added and the solution is diluted to 5 litres.

Starch solution. 1 g soluble starch is dissolved in 100 ml distilled water by warming until the solution becomes clear.

Thyodene indicator (Purkis, Williams Ltd., 60 Brewery Road, London N7). This indicator is better than the starch solution, because the sample remains clear during the whole titration and the end point is easily detected. When using the starch solution the sample mostly becomes turbid.

16.4.3 Equipment

- Calibrated burette, for instance a 12 ml Derona burette with an automatic zero adjuster, or a motor-driven piston burette.

- Automatic syringe pipettes, piston pipette or similar. As the reagents have to be added swiftly, common types of pipettes with or without suction devices should be avoided.

- Winkler bottles, volume about 100-130 ml, carefully calibrated. These may be substituted by common 50 ml glass bottles with ground stoppers, if these stoppers are long enough to extend through the bottle neck and into the body of the bottle, thus eliminating the risk of trapping air bubbles in the bottle.

16.4.4 Procedure

The sample should if possible be drawn from water samplers of plastic or plastic-coated metal. This should be done as soon as possible after the hydrographic cast (sampling). The oxygen sample should be the first sample drawn from the water sampler. For this purpose a rubber tubing is fitted to the outlet of the sampler. A glass tube about 10 cm long is connected to the tubing. The glass tube is dipped to the bottom of the Winkler bottle and at least one volume of sample water is allowed to flow through the bottle before filling with the sample. The water should flow at a moderate speed, avoiding air bubbles. (Air bubbles trapped in the rubber tubing are removed by squeezing.) Then the tube is carefully removed from the bottle which is filled to the top. With automatic syringe pipettes the reagents manganous sulphate and hydroxide-iodide are added simultaneously. The syringes have long plastic tips, extending deep into the bottle. 1 ml of each reagent is added to the sample volume 100-130 ml. (To the smaller bottle 0.5 ml of each is added.) The Winkler bottle is carefully closed with the stopper avoiding the trapping of air bubbles, and the bottle is vigorously shaken.

When the precipitate has settled to about half way down the bottle, 1 ml sulphuric acid (1 + 1) is added with an automatic syringe. The bottle is closed again, avoiding air bubbles, and shaken until the precipitate is dissolved. Now the bottle should be stored in a dark place and titrated after a maximum time of one hour. If the analysis has to be delayed, the

bottles should be stored with the oxygen precipitated. The acid should by no means be added if the bottles have to be stored for a long time. If dissolved, the iodine will evaporate, passing the glass stopper. If the samples have to be stored for several days, or until the ship arrives at the home port, the Winkler bottles should be stored in glass or plastic jars filled with sample water. The bottles should be completely submerged, and the jars closed (it might be a good idea to store the bottles upside-down in the jars).

The dissolved sample is quantitatively washed down into a wide-necked 300 ml Erlenmeyer flask and titrated with standard thiosulphate solution, until a very pale straw colour remains. The titration must be carried out at an even speed. Three drops of starch solution - freshly prepared - (or Thyodene indicator) are added and the titration is continued until the solution is colourless. If the thiosulphate is added too slowly at the equivalent point, there will always be a slight colour in the sample due to oxidation.

16.4.5 Calculation of the oxygen concentration and saturation

a = volume of the thiosulphate consumed in ml

N = normality of the thiosulphate solution

V = volume of the Winkler bottle in ml

then O_2 mg/l = $\frac{a.N.32000}{4.(V-2)}$

If the small sample bottle (50-60 ml) is used the volume of each added reagent is 0.5 ml. In this case the (V-2) in the formula is replaced by (V-1).

The quantity mg/l is normally used in analyses of fresh waters, sewage waters, etc. However, in oceanography ml/l is the preferred term. This quantity is obtained by dividing the mg/l value by 1.43 or substituting the constant 32000 with 22393 in the formula above.

At any given temperature and salinity, a water is saturated with oxygen when it has dissolved a certain, specified quantity. This quantity is referred to as the saturation concentration or 100 percent saturation. Thus, any oxygen concentration found by analysis can be expressed as a percentage of the saturation value instead of ml/l or mg/l. The saturation concentration of the water is obtained from a standard table (for sea water, Unesco, 1974; for fresh water, Montgomery et al., 1964) when the temperature and salinity of the sample are known. Instead of the standard tables a nomogram can be used.

Standardization of the thiosulphate solution. The exact titre of this solution can be determined by titration against iodate ions in acid solution:

$$IO_3^- + 5I^- + 6H_3O^+ \longrightarrow 2I_3^- + 9H_2O$$

$$6S_2O_3^{2-} + 2I_3^- \longrightarrow 3S_4O_6^{2-} + 6I^-$$

Thus 1 M iodate is equivalent to 6 M thiosulphate.

Analytical grade potassium iodate (KIO_3, molecular weight 214.04), is dried at 180°C to constant weight. Exactly 1.1891 g KIO_3 is dissolved in distilled water and diluted to 1 000 ml. 5 ml of this 0.0333 N solution is added to a glass-stoppered Erlenmeyer flask (250 ml) containing 40 ml distilled water, 1 ml of the sulphuric acid (1 + 1) solution, 1 ml manganous and 1 ml sodium reagent. After five minutes the solution is titrated with the thiosulphate solution as described for the samples. The reagent blank is obtained by omitting the addition of iodate and then repeating the procedure. The addition of starch should give no, or only a faint, blue colour.

O_2 4/H_2S 1

N = the titre of the thiosulphate solution

a = ml KIO_3 solution

y = the normality of the KIO_3 solution

b = ml thiosulphate solution consumed, corrected for blank

$$N = \frac{a.y}{b}$$

NOTE:

The amount of sodium iodide has to be at least 600 g/l, according to Carritt and Carpenter. Potassium iodide may as well be used, but may be more difficult to dissolve in the concentration used.

Potassium hydrogen iodate, $KH(IO_3)_2$, may as well be used as standard titre substance.

It is very important that the whole sample is titrated. Due to the losses of iodine, it is impossible to take out a representative aliquot.

16.5 Determination of hydrogen sulphide

16.5.1 Introduction

Sulphide is found in anoxic waters, where it is formed by microbiological reduction of sulphate ions. The method described here, depending on the formation of methylene blue from dimethyl p-phenylene diamine, is a simple application of a well established colorimetric method for sulphide.

The method is based on the following principle: The acidified sample is allowed to react with dimethyl p-phenylene diamine, with ferric ions as catalyst. A complex oxidation and substitution takes place, resulting in the quantitative incorporation of any sulphide-sulphur present into a heterocyclic dye called methylene blue. The absorption of light by the sample is measured before or after dilution in 1, 5 or 10 cm cells. The method is as nearly sensitive as is theoretically possible, and is for direct determination applicable to concentrations up to about 100 µgat/l – or about 3.2 mg/l (see paragraph 16.5.4). At higher concentrations of hydrogen sulphide the sample has to be diluted with oxygen-free distilled water prior to the analysis.

16.5.2 Reagents

N,N-dimethyl p-phenylene diamine dihydrochloride, p.a., $(CH_3)_2NC_6H_4.NH_2.2HCl$ (1,4). 1 g is dissolved in about 6 N hydrochloric acid to 500 ml. This acid may be prepared by diluting concentrated HCl (37 percent, sp. gr. 1.19) with an equal amount of distilled water.

Ferric chloride, p.a., $FeCl_3$. 8 g is dissolved in about 6 N hydrochloric acid (prepared as above) to 500 ml.

Oxygen-free distilled water. A suitable volume of distilled water is boiled for 30 to 60 minutes. Nitrogen gas may be bubbled through the water during the boiling. As the water cools down to room temperature, nitrogen gas must be bubbled through. This water is difficult to store, so it should be prepared just before use.

Sulphide stock solution. A solution of sodium sulphide is prepared with oxygen-free distilled water. Crystals of $Na_2S.9H_2O$ p.a. are quickly washed with distilled water by squirting from a washing bottle. The crystals are dried with filter paper and placed in a pre-weighed, glass stoppered, weighing glass. 0.750 g is weighed on an analytical balance and dissolved in oxygen-free distilled water (added with aid of a siphon) to 1 000 ml in a volumetric flask.

Sulphide working solution. Siphon a suitable volume of oxygen-free distilled water into a 500 ml volumetric flask. Into this is pipetted 25 ml of the sulphide stock solution and oxygen-free distilled water is added to the mark. This solution contains approximately 5 µg/ml of sulphide, S^{2-} (about 0.156 µgat/ml). For the greatest accuracy, the concentration is determined by titration. The solution is stable for only 15-30 minutes.

Sodium thiosulphate solution, 0.02 N. Same solution as for the determination of dissolved oxygen. 5 g $Na_2S_2O_3.5H_2O$ p.a. is dissolved in 1 000 ml distilled water. Add 5 ml isobutyl alcohol before diluting to the full volume of the volumetric flask.

Potassium hydrogen iodate solution. Weigh out accurately 1.2998 g $KH(IO_3)_2$ p.a. dried at 105° C for one hour. Dissolve in distilled water and dilute to 1 000 ml. This solution is 0.04 N and very stable.

Sulphuric acid (1 + 1) solution. Same reagent as for the dissolved oxygen determination. One volume of acid is carefully mixed with one volume of distilled water under constant cooling and mixing.

Starch solution. 1% or Thyodene indicator as for the dissolved oxygen determination.

Potassium iodide, KI, p.a. crystals.

16.5.3 Equipment

- Winkler bottles.
- Spectrophotometer or filterphotometer with filter at or close to 670 nm. 1 cm and 5 cm or 10 cm cells.
- Automatic syringe pipettes.

16.5.4 Calibration

Standardization of the thiosulphate, determination of the factor (f). Add 1 g potassium iodide and 2 ml sulphuric acid (1+1) to an Erlenmeyer flask with a ground stopper, containing approximately 150 ml distilled water. Mix well and add 10 ml of the potassium hydrogen iodate solution by means of a pipette and stopper the flask. Swirl gently and allow the iodine liberation to proceed (away from direct sunlight) for 2-4 minutes. Titrate the iodine with the thiosulphate solution to a pale straw colour. Add two drops of the starch solution as indicator and complete the titration until the disappearance of the blue colour. A blank is run to check the reagents. This is done by repeating the process, but omitting the hydrogen iodate. If v is the millilitres of thiosulphate consumed, then:

$$10 - 0.04 = f \cdot v \cdot 0.02 \quad \text{and}$$

$$f = \frac{20}{v}$$

The mean value of (f) should be calculated after at least three runs, differing not more than 0.05 ml.

Standardization of the sulphide working solution. This is carried out within minutes after preparation of the working solution and at the same time as the preparation of the photometric standard samples.

Take six Erlenmeyer flasks with ground stoppers and add to each about 10 ml distilled water and 1-2 g potassium iodide. Pipette into each flask 10.00 ml hydrogen iodate solution. Add 1.0 ml sulphuric acid (1+1) into each flask. Into three of the flasks pipette 50 ml of the sulphide working solution and to the other three add about 50 ml distilled water. Set all flasks aside in a cool place whilst the colorimetric standardization is commenced and then titrate the contents of the flasks with thiosulphate using starch indicator.

H_2S 3

A = mean of the titrations of three solutions with no added sulphide, in ml.

B = mean of the titrations of the three solutions containing sulphide, in ml.

f = factor of the thiosulphate

(the individual titrations constituting a triplicate should agree to within 0.05 ml).

$$H_2S \text{ ml/l} = \frac{221.40 \cdot f \cdot (A-B)}{50}$$

To convert this to µgat/ml H_2S (or rather µmoles, if the result is reported as hydrogen sulphide instead of sulphide, S^{2-}), the number is divided by 22.140:

$$H_2S \text{ µgat/ml} = \frac{10 \cdot f \cdot (A-B)}{50}$$

Photometric standard samples. From the working solution the following standard series is prepared.

To 100 ml measuring flasks or volume calibrated Winkler bottles the following volumes of working solution are added by means of a pipette or burette (Table III):

Table III

20 ml	=	100 µg S^{2-}	=	31.2 µgat/l S^{2-}
16 ml	=	80 " "	=	25.0 " "
12 ml	=	60 " "	=	18.7 " "
8 ml	=	40 " "	=	12.5 " "
4 ml	=	20 " "	=	6.3 " "
0 ml	=	0 " "	=	0.0 " "

These concentrations correspond to the solution with 5 µg/ml. Thus they have to be corrected according to the value found by titration.

If Winkler bottles are used the concentration values given above have to be recalculated accordingly for the calibration graph described below.

With the aid of a siphon the bottles are filled up with oxygen-free distilled water to the 100 ml mark, or in case of calibrated Winkler bottles, to the neck. In the latter case a correction for the volume has to be made for each bottle.

As soon as a bottle is filled up, 1 ml of each reagent is added with an automatic syringe pipette and the contents of the bottle are mixed well. After 60 minutes, the samples are measured against the blank, at 670 nm in 1 cm and/or 5 cm cells, as suitable. From the results a calibration graph for each of the cell lengths is prepared on millimeter paper. The graph should be a straight line and go through origo.

If higher concentrations are analysed, the graph will start deviating from the straight line at 40-50 µgat/l (about 1.3-1.6 mg/l). The exact point for the start of the deviation depends on the quality of the amine solution. However, if this is taken into account when calculating the analytical results, the graph - and thus the working range of the method - can be extended up to about 100 µgat/l (about 3.2 mg/l).

16.5.5 Sampling and analysis

The samples must be taken with plastic water samplers. If these are not available, metallic ones may be used provided that the inner surfaces are plastic-coated.

An ordinary Winkler bottle is filled with the sample in exactly the same manner as described for the dissolved oxygen determination (16.4). The reagents diamine and ferric chloride are immediately added with pipettes (preferably automatic syringe pipettes) to the sample. Let the pipette tips extend deep into the sample bottle. The stopper is now inserted avoiding air bubbles. The blue colour starts developing in a few minutes and the sample is ready for measurement in a photometer after 30 minutes. However, if the sample contains high concentrations of hydrogen sulphide, one hour must be allowed for full colour development. The colour intensity may be regarded as constant for at least 24 hours.

The colour intensity of the sample is measured against distilled water (or compensated for by reagent blanks) at 670 nm using 1 or 5 cm cells as required.

If the sample contains higher concentrations of hydrogen sulphide than 100 µgat/l (3.2 mg/l) it has to be diluted prior to the analysis. This is done by pipetting a suitable volume of the sample into a measuring flask or Winkler bottle containing some oxygen-free distilled water. The pipette tip should extend below the surface of the water. Then more of the diluting water is added by means of a siphon up to the calibrated volume of the flask or bottle. The reagents are added and the sample is then thoroughly mixed. Account has to be taken of the dilution factor when calculating the result of the analysis.

NOTE: It is difficult to prepare a standard solution of sulphide with a high degree of accuracy. Therefore a systematic error may be included in the analysis. Using the method described, this error will be below 2%.

It seems impossible to obtain a diamine that is not more or less discoloured. However, this does not seem to affect the results. Analyses done at the Fishery Board of Sweden with a one-year old brownish diamine solution produced results which differed only slightly from the results obtained with a freshly prepared solution.

According to Strickland and Parsons (1968) it may be necessary to compensate the absorption value of each sample for the absorption value of a reagent blank. This latter value is obtained by adding reagents to filtered surface water and measuring this against the same filtered water containing no reagents. The absorption value should not exceed 0.5 in a 10 cm cell and should preferably be less than 0.25. At the Fishery Board of Sweden, even using the quoted brownish diamine solution, the blank values have always been negligible.

16.6 Determination of dissolved inorganic phosphorus

16.6.1 Introduction

The phosphate in water is allowed to react with ammonium molybdate, forming a complex heteropoly acid. This acid is reduced by ascorbic acid, to a blue-coloured complex, the light absorption of which is measured in a photometer. Normally this reduction is slow, but by adding a catalyst - in this case antimonyl tartrate - the reduction proceeds swiftly.

This is a modification of the well known single solution method by Murphy and Riley (1962). The use of two different solutions makes the reagents more stable. According to experience from intercalibrations it is necessary to measure the samples of sea water against water from the same depths in order to compensate for the varying turbidity. This turbidity is somewhat changed by the acid in the molybdate reagent. The turbidity blank should for this reason be of the same acidity as the sample. Therefore it is convenient to use two reagent solutions - one containing all components except the ascorbic acid and a second which is the ascorbic acid solution.

PO_4-P 2

No error from salinity will occur in the determinations as this deviation is less than 1 percent. No interferences from copper, iron and silicate occur at concentrations several times higher than those in sea water. Arsenate reacts giving a similar colour, but is regarded as existing in minimal amounts in sea water. Recent results by Johnson (1971) indicate, however, that the concentration of arsenate is higher than was believed. In periods of high primary production - and hence low concentrations of phosphate - arsenate and phosphate levels are said to be equal and sometimes arsenate may be dominating.

16.6.2 Storing of samples

The analysis should be commenced as soon as possible, preferably within half an hour, and definitely before two hours. Samples should be kept in a cool dark place and not warmed to room temperature until the time of analysis. If the analysis has to be delayed for more than about one hour, store the samples in the refrigerator. On short trips it may be possible to add the reagents to the samples and perform the measurements ashore. There is conflicting evidence concerning the effect of different preservation methods and the fate of phosphate molecules in glass and plastic bottles. No entirely satisfactory preservation method is known.

16.6.3 Reagents

Sulphuric acid, 9.1 N. 253 ml of concentrated sulphuric acid (sp. gr. 1.84) is, under cooling and continuous mixing, carefully poured into a suitable volume (600 ml) of distilled water. After cooling the volume is adjusted to 1 000 ml with distilled water.

Ammonium heptamolybdate solution, 0.073 M. Dissolve 9.0 g of the salt $(NH_4)_6Mo_7O_{24}.4H_2O$ in distilled water and dilute to 100 ml. A new solution should be made if any precipitation occurs.

Acid - molybdate reagent. 200 ml of the sulphuric acid mentioned above is mixed under continuous stirring with 45 ml of the molybdate solution. Finally 5 ml of the tartrate solution is added. If stored cold in a dark bottle the reagent is stable for several months.

Ascorbic acid solution, 0.4 M. 7.0 g of ascorbic acid, $C_6H_8O_6$, is dissolved in distilled water and diluted to 100 ml. If stored cold in a dark bottle, the reagent is stable for some weeks.

For preservation, 0.05 g of EDTA (ethylene diamine tetra-acetic acid) and 1 ml formic acid, HCOOH, may be added.

Phosphate stock solution. Potassium dihydrogen phosphate is dried over concentrated sulphuric acid or at 100°C. 0.3403 g of KH_2PO_4 p.a. is dissolved in distilled water and diluted to 1 000 ml in a volumetric flask. Some drops of chloroform are added for preservation. This solution contains 2.5 µgat/ml (or 77.4 µg/ml) PO_4-P. The solution is stable for many months if kept in a refrigerator.

Phosphate working solution. 10 ml of the stock solution is diluted with distilled water to 1 000 ml in a volumetric flask. 1 ml contains 0.025 µgat (or 0.77 µg) PO_4-P. This solution should not be stored for future use.

16.6.4 Equipment

- Spectrophotometer or filter photometer with filter at or close to 882 nm. Cells of 5 cm and/or 10 cm length.

- Glass stoppered test tubes, 30-50 ml volume, or other suitable containers.

- Kipp pipette (automatic dispensing pipette) of 35 ml or 25 ml volume.

- Automatic syringe pipettes of 1 ml or 2 ml volume.

These last two types of pipettes are not necessary, but they make the routine handling of a great number of samples easier.

16.6.5 Calibration

A series of working standards are prepared from the phosphate working solution by dilution with distilled water. If 50 ml volumetric flasks are used, Table IV can be used.

Table IV

| | | |

PO4-P 4/tot P 1

The corrected absorbance is used for evaluation of the result, using the calibration graph described in paragraph 16.6.5.

NOTE: The use of a turbidity blank may be omitted in case of high concentrations of phosphate and - at the same time - insignificant turbidity. However, to test the significance of the turbidity, it is recommended to analyse at least some samples with and without a turbidity blank.

If 5-cm cells are used instead of 10 cm, the sample volumes may be reduced to 25 ml and the reagent volumes to 0.7 ml.

16.7 Determination of total phosphorus

16.7.1 Introduction

All phosphorus compounds occurring in natural and sewage waters may be oxidized with peroxydisulphate to phosphates. If the water contains chloride ions, the acid oxidation will result in development of chlorine gas. This gas has to be destroyed (by sodium sulphite) in order to prevent bleaching of the colour complex. The phosphate is then determined as described for dissolved inorganic phosphates.

Three different modifications of the oxidation procedure are described below, all of which have in common that the oxidation takes place in the presence of 1 percent of peroxydisulphate.

16.7.2 Storing of samples

As for the dissolved inorganic phosphates, there is conflicting evidence concerning the best way to store the samples in case a determination cannot be done immediately after sampling. The storing of total phosphorus samples may be regarded as less critical, however, as any transformation between organic and inorganic compounds does not affect the result; after the oxidation all phosphorus is present as inorganic phosphorus.

It is essential to prevent the adsorption of compounds to the sides of the sample bottle, as this will cause too low results. Adsorption to the sides may be prevented by adding a suitable amount of sulphuric acid (1 ml of 8 N acid per 100 ml of sample) to the sample.

Another way to deal with the adsorption problem is to store the samples in the small oxidation bottles (see paragraph 16.7.5). As the sample is never transferred between storage and analysis, no loss of material will result from the adsorption. All phosphor-containing compounds seem to be oxidized regardless of whether they are dissolved or adsorbed.

16.7.3 Reagents

In addition to the reagents required for the determination of dissolved inorganic phosphates, the following are needed.

(1) Potassium peroxydisulphate, $K_2S_2O_8$, crystals, reagent grade.

(2) Sodium sulphite solution 1 M, Na_2SO_3, anhydrous crystals, reagent grade: 12.6 g is dissolved in distilled water and diluted to 100 ml. Store the solution cold and prepare a new one daily or every second day, as the solution is unstable.

16.7.4 Equipment

- The equipment used for the determination of dissolved inorganic phosphates.
- Special spoon. This is easily made of a plastic bar about 5 x 12 x 100 mm. A hole is drilled near one end of the bar with such dimensions that it will contain 0.35 ± 0.05 g of potassium peroxydisulphate when the excess is levelled off with a spatula.

- Depending on the chosen procedure for the oxidation, heating mantles or a pressure cooker, flasks or bottles are needed. These items are described in connexion with the different procedures.

16.7.5 Procedure

Boiling in open flasks. To a 35 ml sample in a 100 ml Erlenmeyer flask (of resistant glass) 0.35±0.05 g of potassium peroxydisulphate is added. This is done with the special plastic spoon. The spoon is filled and then the excess reagent is gently swept off with a spatula. This is important because if excess reagent is added, the pH in the final sample will be too low, thus giving an incomplete colour development.

The sample is boiled in a small electric heating mantle for about 40 minutes or until the volume is 5-7 ml. After cooling down to room temperature, the solution is diluted to 35 ml again, with distilled water.

Then the sample is analysed as described for dissolved inorganic phosphates, paragraph 16.6.6. No turbidity blanks are needed after this oxidation.

If 5 cm photometric cells are used instead of 10 cm, 25 ml of sample can be used. In this case, however, it must be noted that 0.25±0.05 g of potassium peroxydisulphate should be added.

If the samples have been preserved with acid, they must be neutralized with sodium hydroxide prior to the oxidation.

Boiling at pressure in open flasks. A common kitchen pressure cooker is used; in this case the temperature will rise to around 120 °C. The same sample volume and amount of peroxydisulphate are used as above, but 50 ml Erlenmeyer flasks are used as the boiler volume is restricted. The openings of the flasks are covered with plastic or aluminium foil. After boiling for 30 minutes at pressure, the samples are cooled to room temperature and the volume of the samples is adjusted to 35 ml with distilled water.

Then the samples are ready for the addition of reagents as described in paragraph 16.6.6.

If the samples have been preserved with acid, they must be neutralized with sodium hydroxide prior to the oxidation.

This method should be used for fresh water samples only, as brackish and sea waters evolve so much free chlorine during the oxidation that the boiler is destroyed in the long run. This is avoided in the following method.

Boiling at pressure in closed bottles. When closed bottles are used the stopper must be properly tightened. Suitable bottles are 50 ml "Rollrandflaschen mit Bügelverschluss" made by Schott Gen., Mainz, No. 4176b (Figure 12). The iron loops are swiftly corroded by the free chlorine produced during the oxidation. The loops have thus to be replaced by new ones made of stainless steel. The gaskets too have to be replaced by others made of silicon rubber. They can easily be cut from a tube with about 16 mm outer diameter and 10 mm inner diameter.

Another suitable bottle is made by Sovirel (No. 362-33, 50 ml). The screw caps do not withstand chlorine, and have to be substituted for others of polypropylene (Nalgene No. 2150, size 28).

Sample volume, amount of peroxydisulphate and boiling time should be the same as in paragraph 16.7.5. The oxidant ought to be dissolved in the sample before boiling. The sample volume will be constant in these bottles if they are properly closed.

tot P 3

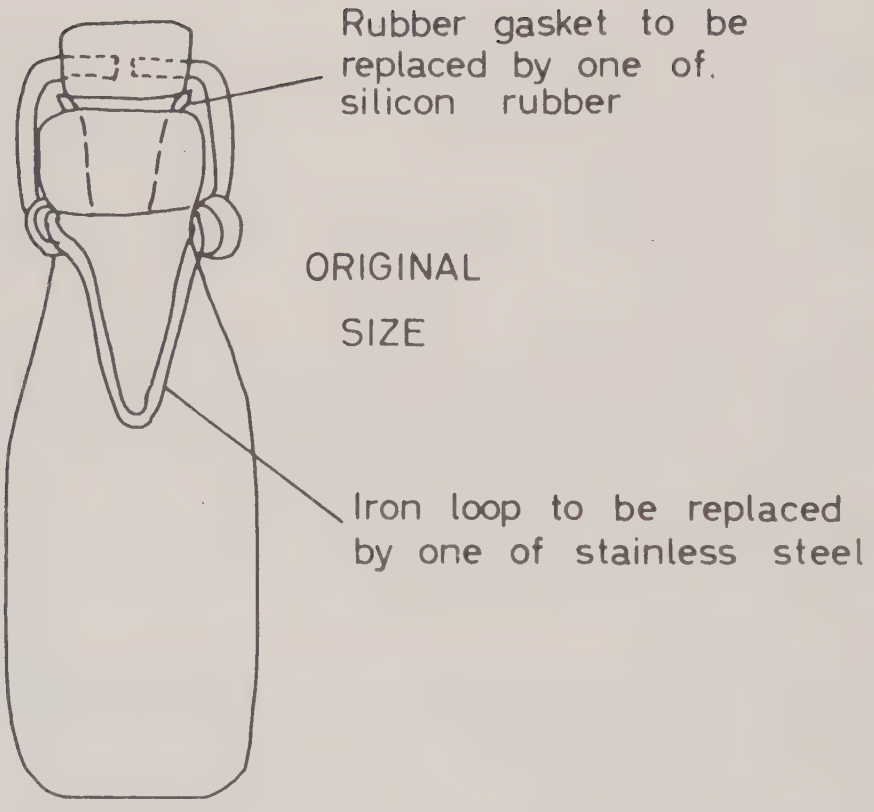

Figure 12. Bottle for analysis of total nitrogen and total phosphorus

(Schott Gen. 4176b, Rollrandflasche mit Bügelverschluss 50 ml, available also from Jena in GDR)

To keep the bottles clean the samples are, after the boiling, transferred to test tubes, where the analysis is completed as follows:

To destroy the free chlorine 0.5 ml of the sulphite solution is added. (In any case, not more than this volume should be added, because that will give a wrong pH value.) Mix well and let the sample stand for ten minutes in the unstoppered test tube. Then add 1 ml ascorbic acid and mix again. After another five minutes the acid - molybdate reagent is added and the sample is mixed once more.

The samples are then measured in the photometer as described in paragraph 16.6.6.

This reversed order of adding reagents facilitates the removal of residual chlorine. If not all the chlorine has been destroyed, bleaching of the molybdenum blue complex may occur and hence too low results are obtained.

NOTE: If the samples have been preserved with acid, they must be neutralized with sodium hydroxide prior to the oxidation.

16.7.6 Evaluation of the results

Calibration, dilution, range of determinations and precision. The calibration for the determination of dissolved inorganic phosphates was described in paragraph 16.6.5. This calibration is normally sufficient for the determination of total phosphorus too, as the oxidation with peroxydisulphate is complete. However, to check for losses due to leakages from the bottles, spattering, etc., some standard samples should be run from time to time.

It has been pointed out in connexion with the oxidation procedures, that acidified samples have to be neutralized prior to the analysis. If this neutralization is carried out before measuring the portion to be analysed (or even after, when closed bottles are used for the oxidation) the sample is diluted to some extent. Thus the volume of the hydroxide added has to be recorded and the resulting dilution corrected for when the analytical result is calculated. If, on the other hand, the hydroxide is added to the measured sample portion in the open oxidation containers, no dilution has to be corrected for. In these cases the volumes are adjusted to the original 35 ml after the oxidation.

The maximum concentration of phosphorus which can be directly determined is 28 µgat or 870 µg per litre. The precision is very good and the reproducibility is in most cases better than 0.02 µgat or 0.7 µg per litre. For concentrations less than 1 µg per litre, an enrichment of the reduced complex with an organic solvent is recommended.

Disturbance from silicate. It is a well known fact that the blue phosphomolybdenum complex formed with ascorbic acid is stable for hours. If great amounts of silicate are present, especially in some types of river and lake waters, a blue silicomolybdate complex is gradually formed. With the stated concentrations of sulphuric acid and molybdate, and by measuring the absorption after five minutes, up to 10 mg of SiO_4-Si per litre can be present without disturbance.

Disturbance from arsenate. The arsenate ion gives with sulphuric acid, molybdate and ascorbic acid, a blue complex. However, the reaction is rather slow, being complete after one hour. By measuring the absorption after five minutes the disturbance from arsenate is avoided. It may be mentioned that with stannous chloride as reductant the blue arsenate complex is formed immediately.

Influence of the concentration of the sulphuric acid. During the oxidation the peroxydisulphate is almost completely decomposed and one of the products is sulphuric acid. From 350 mg of the oxidant about 120 mg acid are produced, increasing the final concentration to 0.27 N. This increase from 0.2 N can be tolerated, as it has been shown experimentally that with the given concentration of molybdate (0.045 percent) and Sb-ion (0.00068 percent) the normality of the sulphuric acid may vary from 0.12 to 0.30. If the final concentration is

0.35 N, the sensitivity decreases by about 30 percent. As already mentioned this has to be observed if the samples have been preserved with acid.

If the Sb-ion concentration is 0.00068 percent and the H_2SO_4/molybdate ratio (normality/percent) constant, the concentration of acid may be increased to 0.7 N (Murphy and Riley 0.4 N). In 1.0 N acid the sensitivity again decreases by 30 percent.

16.8 Determination of reactive silicate

16.8.1 Introduction

The determination of dissolved silicon compounds in natural waters is based on the formation of a yellow silicomolybdic acid, when a more or less acidic sample is treated with a molybdate reagent. Only silicic acid and its dimer react with molybdate at any appreciable speed and the methods therefore give only the amount of "reactive" silicate, which is probably a reasonable measure of the silicate available to growing phytoplankton.

Depending on pH the yellow silicomolybdate complex exists in two isomeric forms, which differ only in their hydration. The β-silicomolybdic acid which is formed below a pH value of 2.5 is rather unstable, but has a higher molar absorbance than the α-acid.

In early work silicate was determined by visual or photometric estimation of the β-complex. The literature has been reviewed by Mullin and Riley (1955), and in 1968 a careful study of the optimum conditions for this technique was performed by a research group at the Sagami Chemical Center in Japan. In their modified method the colour of the complex is stable from 5 to 20 minutes at room temperature, in both distilled water and sea water; the salt effect is diminished to 3 percent.

Grasshoff (1964) has developed a method based on the formation of the α-isomer at a pH of 3.7 to 4.0, and states that the colour in sea water is stable (after two hours) for days.

Since both of the yellow silicomolybdic acid isomers are rather weak in colour, several methods have been developed in which they are reduced to intensely coloured, blue complexes. A wide range of inorganic and organic reducing agents have been used. A mixture of metol and sulphite is commonly used. In the method described here ascorbic acid acts as the reductant.

The sensitivity and capability of this method is as follows:

The molar absorption of the coloured complex is about 22 200. This implies that if the concentrations are given as µgat/l, the absorbance 1.00 is equal to 45 µgat/l Si with a 1 cm cell used for the measurement. With this cell length up to about 80 µgat/l (about 2 250 µg/l) of Si can be determined (absorbance about 1.7). With a 10 cm cell, 0.045 µgat/l (about 1.26 µg/l) of Si can be determined. As this corresponds to an absorbance of 0.010, it can be regarded as the limit for direct determination.

16.8.2 Sample storage

Samples of sea water for silicate determination should not be stored in glass bottles for more than a short while prior to analysis and it is best, therefore, to transfer samples directly into containers of polyethylene or waxed glass. To minimize the effect of diatom multiplication, store samples in the dark and for no longer than a day prior to analysis. Conflicting opinions and evidence exist as to whether samples can be stored for long periods or not, and if stored, whether they should be deep frozen or not. If deep frozen, the silicate concentration can be expected to decrease by at least 10 percent. Thus it is recommended to perform the analysis as soon as possible after sampling. If the samples have to be stored, the tolerable storage time ought to be investigated and the possible size of the error estimated.

16.8.3 Reagents

All solutions should be prepared from reagent grade (pro analysi) chemicals, using good quality distilled water. The solutions should preferably be stored in plastic bottles rather than glass bottles.

Ammonium heptamolybdate solution 0.16 M. Dissolve 49.5 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and dilute to 250 ml. As the salt dissolves slowly, moderate heating may be applied.

Sulphuric acid solution 7.3 N. 198 ml H_2SO_4 (concentrated, sp. gr. 1.82) is carefully poured, under constant cooling and mixing, into distilled water and finally diluted to 1 000 ml.

Molybdate reagent. Equal volumes of the molybdate solution and the dilute sulphuric acid solution are mixed.

Oxalic acid solution 0.7 M. Dissolve 63 g $C_2O_4H_2$ and dilute to 1 000 ml.

Ascorbic acid solution 0.1 M. Dissolve 4.4 g $C_6H_8O_6$ and dilute to 250 ml. Stored cold in a brown bottle, the reagent is stable for weeks.

Silicate stock solution. Sodium silicofluoride, Na_2SiF_6 p.a. is dried over concentrated sulphuric acid to constant weight. Weigh in 0.4701 g of the salt and dissolve in distilled water in a plastic beaker. Transfer to a 500-ml volumetric flask and dilute to the specified volume with distilled water. Finally, transfer to a plastic bottle. Some authors report this solution to be stable indefinitely (provided that evaporation is prevented), but for safety fresh solution may be prepared for every calibration. 1 ml contains 5.0 µgat Si (140.5 µg Si).

Silicate working solution. 50 ml of the stock solution is diluted with distilled water or synthetic sea water of appropriate salinity (see below) to a final volume of 500 ml. 1 ml contains 0.50 µgat (14.05 µg) Si. This solution should be used at once.

Synthetic sea water. 25 g sodium chloride, NaCl p.a., and 8 g magnesium sulphate heptahydrate, $MgSO_4\cdot 7H_2O$ p.a. are dissolved in distilled water for every litre of solution. This water must be stored in a plastic bottle. For analytical purposes it is equivalent to sea water of 28 percent salinity. For calibration work it may be diluted to the desired salinity. The silicate concentration should be below 1-2 µgat/l Si (28-56 µg Si).

16.8.4 Equipment

- Test tubes. Stoppered tubes of glass may be used but plastic ones are preferred (cheap tubes made from polystyrene are commercially available). Small plastic bottles (25-50 ml) may also be used.

- Automatic syringe pipettes (3 pcs.), capacity 2 ml

- Spectrophotometer, or filter photometer with the filter at or close to 810 nm. If this is not provided, use a filter having a maximum transmission above 700 nm; in this case the sensitivity is reduced and a slight deviation from the Lambert-Beer's law may occur. Cells of 1, 5 and 10 cm length as required.

16.8.5 Calibration

From the working solution a series of working standards are prepared by diluting with distilled water or synthetic sea water in volumetric flasks. The table below may be used.

For routine analysis in sea water it is normally sufficient to prepare the calibration solutions in synthetic sea water of a salinity representing a mean value of the salinities in the working area. However, for the greatest accuracy, it should be more convenient to calibrate in distilled water and then correct for the salt error.

Silic 3

ml of working solution/100 ml (dilute with synthetic sea water or distilled water)	µgat/l Si	µg/l Si
0.0	0.0	0.0
0.5	2.5	70.2
1.0	5.0	140.5
2.0	10.0	281
5.0	25.0	703
10.0	50.0	1 405
20.0	100.0	2 809
25.0	125.0	3 512
30.0	150.0	4 214

Some of the standards are chosen depending on the expected concentrations in the samples. Samples are pipetted from the working standards to the test tubes. The distilled water or synthetic sea water of the chosen salinity can be used as the blank. The reagent blank must be prepared together with every calibration.

The blank sample compensates for the silicate content in the reagent as well as in the distilled water (or synthetic sea water) used for the dilution of the standards. However, the same blank value cannot be used for the analysis of the natural water samples. In this latter case a reagent blank has to be used.

The absorbances are plotted versus concentration on a millimetre paper. The result should be a straight line over the entire range of concentration.

16.8.6 Procedure

To 35 ml of the sample in a plastic test tube 1.0 ml of the molybdate reagent is added. After 10 to 30 minutes (for fresh water) or 5 to 10 minutes (for sea water), 1.0 ml of the oxalic acid solution is added followed immediately by 1.0 ml of the reductant (ascorbic acid). Swirl gently during the reagent additions. After 30 minutes the absorbance of the sample is measured against distilled water in a cell of suitable length at a wave-length of 810 nm. Correct the measured absorbance by subtracting that from the reagent blank and the possible reference sample.

The reagent blank is prepared by carrying out the above procedure using 35 ml of distilled water. The absorbance is denoted A_1. Repeat the determination, but with only 0.5 ml of the molybdate reagent. In this case measure the absorbance after one hour (A_2).

Absorbance of reagent blank = $2(A_1 - A_2)$

The absorbance caused by the silicate content of the reagents only is thereby obtained. This reagent blank should be performed every time any of the reagent solutions is prepared. In addition, it is advisable to check the reagent blank from time to time.

For the most precise estimate of low amounts of silicate using a 10 cm cell, a reference absorbance should be measured for every sample to compensate for its natural turbidity. The reference solution is prepared by adding 3 ml of 0.25 M sulphuric acid solution to 35 ml of sample. Thus the absorbance of the sample is:

$$A_{sample} = A_{measured} - A_{reference} - A_{reagent\ blank}$$

The silicate concentration is evaluated using the calibration graph described above. However, for the most precise work in saline waters a correction must be applied for the salt error.

The salt error. The dissolved salts of sea water reduce the final colour intensity appreciably. It has been found that the variation of the salt error is a linear function of salinity and may be calculated from a formula, for example:

$$A_{true} = A_{observed} \cdot (1 + 0.0028 \cdot S‰)$$

However, these figures cannot be recommended for general use. Because of the varying quality of reagents, etc., every laboratory should construct a correction formula of its own, by calibrating at several salinities and then calculating from the observed variations of the absorbances.

Effect of the oxalic acid. The oxalic acid is added for two reasons:

(a) to avoid the reduction of any excess molybdate reagent. The stability of the silicomolybdic acid in the presence of oxalic acid is limited, and the reductant is therefore added immediately after the acid;

(b) to eliminate the influence of any phosphate present. Trials have been made with up to 10 µgat/l (about 310 µg/l) of P and no increase in colour intensity was observed.

Time for colour development and colour stability. For up to about 30 µgat/l (about 840 µg/l) of Si, the colour reaches its maximum after about 30 minutes and is then - provided any evaporation is prevented - stable for several days. However, for practical purposes it may be advisable to regard the coloured complex as being stable for several hours. For higher concentrations, up to an absorbance of 1.7 (1 cm cell), the intensity increases extremely slowly after 30 minutes, only about 0.1 percent per hour.

16.9 Direct determination of ammonia

16.9.1 The indophenol blue method (method preferred for sea water)

16.9.1.1 Introduction

This method. which is specific for ammonia, is applicable to all kinds of natural waters and is the recommended method for sea water. It is four times as fast as the Nessler reaction. "Ammonia" here refers to the sum of ammonia and ammonium ions, because the original proportions of them in a water sample are pH dependent.

The method is based on the following principle:

In a weakly alkaline solution ammonia reacts with hypochlorite to form monochloramine, which in the presence of phenol, catalytic amounts of nitroprusside ions and an excess of hypochlorite yields indophenol blue. By increasing the concentrations of the reagents used in this method, labile organic nitrogen compounds may also react, while the reaction time is decreased. The reaction mechanism leading to the coloured complex is complicated and not yet fully understood. Probably, a quinone chlorimide is formed in an intermediate step. The intensity of the coloured complex is measured in a photometer.

16.9.1.2 Sample storage and preservation

The samples may not be preserved with sublimate ($HgCl_2$). Acid preservation is possible, but in that case every sample must be carefully neutralized with sodium hydroxide before the analysis is started, and corrections made for the ammonia content in acid and hydroxide. This makes the analysis uncertain and the acid preservation should be avoided if possible.

Preferably, the samples should be analysed within a short time after the sampling, and any necessary storage should be in a dark, cold place.

Polluted waters are often turbid and may be filtered through washed glass wool filters. However, such waters frequently contain high concentrations of ammonia and may therefore be diluted before the analysis. Any residual turbidity can be compensated with a similarly diluted sample in the reference cell. Thus filtration should be avoided if possible.

16.9.1.3 Reagents

"Ammonia-free" water. There is not a standard procedure for the preparation of water with a very low ammonia content, as the results obtained depend on the equipment and working facilities as well as the quality of the water used. Each laboratory has therefore to find the method that gives the most satisfactory result.

Deionized water may sometimes be used, with or without subsequent distillation, but it must be remembered that some ion exchange resins may leach out ammonia containing organic substances.

Ordinary distilled water may be used after a second distillation. In this second step, 2 ml concentrated sulphuric acid and 1 g potassium peroxodisulphate ($K_2S_2O_8$) are added per litre. Freshly distilled, such water should contain less than 0.3 µgat (5 µg) of nitrogen per litre.

Reagent A, phenol - nitroprusside solution. Dissolve 27 g of phenol (pro analysi, p.a.) and 0.300 g of sodium nitroprusside dihydrate ($Na_2Fe(CN)_5NO.2H_2O$ p.a.) in ammonia-free water and dilute to 1 000 ml. Stored cold in a dark glass bottle with a glass stopper, the reagent is stable for some months.

Sodium hydroxide solutions 0.34 M and 0.84 M. These two solutions are used for samples of different salinities.

Dissolve 13.6 g and 33.6 g respectively of sodium hydroxide, NaOH p.a., in ammonia-free water and dilute to 1 000 ml. Store the solutions in glass bottles with plastic stoppers.

Sodium hypochlorite, stock solution. Use a common, commercial solution of hypochlorite (bleaching agent), which usually contains 1.5-1.7 M hypochlorite and 0.4 M sodium hydroxide. The true concentration of ClO^- is determined in the following way:

Dissolve about 0.5 g of potassium iodide (KI) in 50 ml distilled water. Add 1.0 ml of the hypochlorite solution and titrate the liberated iodine using a 0.10 M thiosulphate solution. This titration gives the concentration of chlorine and chlorite. If the consumption of thiosulphate is greater than 1 ml, the hypochlorite solution has become too oxidized and should not be used. If less than 1 ml is consumed, some drops of concentrated hydrochloric acid (HCl) are added and the titration is continued (using starch as indicator) until the sample is colourless.

The consumption (in ml) of thiosulphate in this second titration multiplied by 3.54 gives the amount of hypochlorite-chlorine (in mg) in 1 ml of the hypochlorite solution.

Reagent B, alkaline hypochlorite solution, variant I. A volume corresponding to 106 mg of hypochlorite-chlorine is taken from the stock solution and diluted with 0.34 M sodium hydroxide to 100 ml. This solution is used for fresh water and sea water with a salinity not exceeding 20‰. For sea water of greater salinity the dilution is made using the 0.84 M sodium hydroxide. The reagent should be stored dark and cold in a glass bottle with a plastic stopper and is stable for some weeks.

Reagent B, variant II. Instead of using the hypochlorite solution, 0.2 g of Trion may be used. The substance is dissolved and diluted as described above. Trion is the trivial name for 1.3-dichlorocyanuric acid. Depending on the quality of the Trion, this substance may contain a smaller or greater amount of ammonia. Thus a greater blank absorbance is sometimes obtained than with the variant I solution above. However, if this is properly compensated for, no problems will arise from the use of this solution.

Trisodium citrate solution 1.19 M. Dissolve 350 g of trisodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$ p.a.) in ammonia-free water and dilute to 1 000 ml. Store the solution in a glass bottle with a plastic stopper.

Ammonia stock solution. As standard substance, either ammonium sulphate or ammonium chloride of p.a. quality can be used. Before weighing, the salts must be dried to constant weight at 100 °C and then preferably stored in a dessicator.

Dissolve 0.0472 g of $(NH_4)_2SO_4$ or 0.0382 g NH_4Cl in ammonia-free water and dilute to 100 ml. Add a drop of chloroform as preservant. The solutions should be stored in glass bottles. They are stable if protected from direct sunlight and evaporation. Each solution contains 100 µg (7.14 µgat) of NH_3-N per ml.

Ammonia working solutions A (high range) and B (low range). Even if the ammonia concentrations vary considerably in natural waters, one can say as a general rule, that solution A is suitable for calibration in fresh water analysis and solution B is suitable for sea water analysis.

A (high range). Pipette 10.00 ml of the stock solution into a 100 ml volumetric flask and dilute to the mark. The solution contains 10 µg (0.71 µgat) of NH_3-N per ml and it has a limited stability.

B (low range). Pipette 10.00 ml of solution A into a 100 ml volumetric flask and dilute to the mark. The solution contains 1 µg (0.071 µgat) of NH_3-N per ml. This solution has also a limited stability.

16.9.1.4 Equipment

- Test tubes with ground glass (or plastic) stoppers and a volume of 30 ml or more.
- Automatic syringe pipettes of 2 ml capacity (3 pcs.).
- Spectrophotometer or filter photometer with a filter having maximum transmission at (or close to) 630 nm. Photometric cells of 1, 5 or 10 cm length as required.

16.9.1.5 Calibration

Before the test tubes can be used for calibration or analysis, they must be carefully cleaned; it is not sufficient just to wash them, but the following subsequent cleaning procedure must be carried out. To every tube about 25 ml distilled water (not necessarily ammonia-free water) and reagents are added as described in paragraph 16.9.1.6. Let the reaction proceed for at least two hours and shake the tubes from time to time during the reaction period. All ammonia contained in the tubes (dissolved in the water or adhered to the glass wall) will react. Then the tubes are rinsed with ammonia-free water and kept stoppered when not in use. The tubes should not be washed between the different sets of calibrations or analyses, but just rinsed with ammonia-free water.

With the guidance of the table below, the given volumes of either working solution A (high range) or solution B (low range) are pipetted into 100 ml volumetric flasks and diluted to the mark with ammonia-free water.

NH_3-N 4

ml of working solution to be diluted to 100 ml	resulting concentration when working solution A is used		resulting concentration when working solution B is used	
	µg/litre	µgat/litre	µg/litre	µgat/litre
0.00	0	0.00	0	0.00
1.00	100	7.14	10	0.71
2.50	250	17.86	25	1.79
5.00	500	35.71	50	3.57
7.50	750	53.57	75	5.36
10.00	1 000	71.43	100	7.14
12.50	1 250	89.29	125	8.93
15.00	1 500	107.14	150	10.71

Note that the concentration µg/l refers to NH_3-N, which means nitrogen coming from ammonia (and not the concentration of ammonia itself).

From each of the standard concentration solutions above 25 ml is taken (preferably three portions of 25 ml) and transferred to the test tubes. These samples are then analysed as described in paragraph 16.9.1.6.

The following is an example of a calibration series (measured in a 1 cm cell) where the absorbances have not been corrected for the blank value:

µg/l	0	100	250	500	750	1 000	1 250	1 500
A	0.022	0.171	0.397	0.772	1.105	1.440	1.740	2.000

The blank sample here corrects for the absorbance caused by the ammonia in the reagents and also the residual ammonia in the ammonia-free water. After subtraction of the blank absorbance, a calibration graph is constructed, showing absorbance versus concentration.

The calibration graph is reproducible within the entire range for direct determination and it is linear up to a concentration of 500 µg (35.71 µgat) per litre. For concentrations up to 200 µg (14.3 µgat) per litre, a 5 cm cell can be used, and with this cell, concentrations down to 0.6 µg (0.04 µgat) per litre can be determined.

A new calibration is required every time any of the reagents is exchanged for a new solution.

The reagents, especially the citrate solution, contain ammonium ions and hence the absorbance caused by the ammonium in the reagents - the reagent blank - must be determined. To 22 ml of ammonia-free water (preferably three portions of 22 ml), the normal reagent volumes are added - 1 ml of each. To a second set of 22 ml portions, double the reagent volumes - 2 ml of each - are added. After two hours, 3 ml of ammonia-free water is added to each sample in the first set, and the absorbances of all samples are measured immediately. The difference between the mean values of the two sets is the reagent blank. This difference is caused only by the ammonia content of single volumes of the reagents.

For example: the mean of the set with 2 ml of each reagent is A = 0.040 and the mean of the second set is A = 0.022. The difference - the reagent blank - is then 0.018 (for 1 cm cell).

If this is compared with the blank value given in the calibration series it can be noticed that the nitrogen content in the water used for dilution of the calibration samples gives an absorbance of 0.022-0.018 = 0.004, which corresponds to a concentration of about 3 µg per litre.

To obtain the blank value for 5 cm cells, the sample should preferably be measured in that cell. Otherwise the value from the 1 cm cell, multiplied by 5, may be used.

16.9.1.6 Procedure

Before the analysis and between analysis of different sets of samples the test tubes must be cleaned (see paragraph 16.9.1.5).

Calibration solutions, fresh water and sea water with salinity not exceeding 20‰. To a 25 ml sample are added - with mixing after each addition - 1.0 ml of citrate solution, 1.0 ml of reagent A and 1.0 ml of reagent B. Stopper the tubes and keep them in a dark place for at least two hours. If colour develops within five minutes, it may be necessary to repeat the analysis with suitably diluted samples. All dilutions must be made using the ammonia-free water.

The absorbances of the samples are measured in a cell of suitable length at a wavelength of 630 nm. As reference, distilled water is used if the original samples were clear and colourless. Otherwise the sample or a dilution of it (22 ml of diluted sample and 3 ml of distilled water) is used as a reference in the photometer.

The corrected absorbance and the calibration graph is then used for evaluation of the ammonia concentration of the sample.

Sea water with a salinity exceeding 20‰. The procedure is carried out as above, but the hypochlorite solution (reagent B, variant I or II) should be the one which is diluted with the 0.84 M sodium hydroxide. In this case a minimum of four hours must be allowed for the reaction. After the addition of the reagents the pH of the sample will be 10.0-10.1, which is below the required value for a complete colour development. Thus the measured absorbances have to be multiplied by 1.09 before the evaluation of the concentrations is performed.

The reaction and its governing pH conditions. The formation of indophenol via monochloramine is quantitative between pH 10.4 and 11.3. At higher pH values the ammonia is partly oxidized to nitrite and at lower pH values the yield of the coloured complex is less - at pH 8.8 about 50 percent.

At the pH value for quantitative reaction, the Mg and Ca ions are precipitated in sea water as well as in fresh water with an alkalinity exceeding 500 mg/l. In an earlier variant of this method the precipitation was allowed to take place, thus reducing the content of suspended matter and humic substances in the sample. In this variant, the alkaline earth metals are complexed with citrate, which is considered more suitable in a routine method, especially in the presence of higher concentrations of ammonia.

The reaction time. At room temperature the reaction is complete after two hours in fresh water as well as in sea water with a salinity lower than 15‰. In ocean water the reaction requires four hours.

The reaction time may be shortened by using a higher concentration of hypochlorite. This cannot be recommended as some organic nitrogen substances may decompose and form monochloramine, thus giving too high results.

Disturbances. With the recommended concentration of hypochlorite the method is specific for ammonia and other nitrogen compounds do not react. Among inorganic substances, Hg-ions are capable of impeding the reaction. Consequently samples should never be preserved with sublimate ($HgCl_2$). Likewise it has been observed that iron, in concentrations higher than 2 mg/l, causes disturbance.

NH3-N 6

Stagnant water often contains hydrogen sulphide and if its concentration exceeds 2 mg/l a disturbing effect is obtained. In such waters the concentration of ammonia is normally rather high, and thus the disturbance may be eliminated by diluting the samples.

16.9.2 Phenate method

16.9.2.1 Introduction

This method is designed for the determination of ammonia in natural and waste water. However, for the analysis of sea water samples the indophenol blue method (16.9.1) is recommended.

The phenate method is sensitive for interferences from iron (about 2 mg/l), hydrogen sulphide (about 2 mg l) as well as being dependent on mercury, acidity and alkalinity. The interferences are, however, removed by a distillation step, which may be omitted in analysis of unpolluted natural waters.

It must be remembered that if this method is applied without the distillation step the reaction is no longer specific for ammonia. This is because the strong reagents (in comparison with the preceding method) may release ammonia from various labile organic compounds.

This method is based on the following principle:

Free ammonia can be recovered from a sample by distillation at a pH value around 7.4. The ammonia distillate is absorbed in sulphuric acid, where the ammonia is determined colorimetrically. The colorimetric determination is based on the reaction between ammonia and hypochlorite which in the presence of phenol and a manganous salt catalyst forms an intensely blue compound, indophenol. The intensity of the colour is proportional to the ammonia concentration and is measured at 625 nm.

16.9.2.2 Sample storage

The samples may be preserved by adding 1 ml 4M sulphuric acid per 500 ml of sample. Preserved samples may be stored for up to one month at 4°C, but they must always be distilled prior to colorimetric analysis.

16.9.2.3 Reagents

All water used for dissolving chemicals and dilution of samples must be ammonia-free (paragraph 16.9.1.3). The best results are obtained when the water is freshly prepared for each batch of samples. As it is very difficult to prepare water which is entirely free from ammonia, a low residual concentration has to be accepted. If this is properly compensated for, by means of the reagent blank, no problems will arise.

All chemicals should be of reagent grade (pro analysi).

Sulphuric acid 4 M. Add carefully 100 ml concentrated H_2SO_4 to 400 ml water.

Sulphuric acid, 0.1 M. Add carefully 5.6 ml concentrated H_2SO_4 to 900 ml water. Cool and dilute to 1 litre.

Bromothymol blue indicator. Dissolve 0.1 g of dibromothymolsulphonphtalein in 100 ml of 20 percent ethyl alcohol; or dissolve 0.1 g in 8 ml 0.02 N sodium hydroxide solution and dilute to 250 ml. pH range 6.0 (yellow) - 7.6 (blue).

Phosphate buffer solution, pH 7.4. Dissolve 14.3 g potassium dihydrogen phosphate, KH_2PO_4, and 89.5 g dipotassium hydrogen phosphate, K_2HPO_4, and dilute to 1 litre with ammonia-free water.

Manganous sulphate solution, 0.625 mg Mn/l. Dissolve 0.1923 g $MnSO_4.H_2O$ in water and dilute to 1 litre. Dilute 10 ml of this solution to 1 litre.

Acetone.

Methanol.

Sodium phenate solution.

(a) Dissolve 62.5 g phenol, C_6H_5OH, in 20 ml acetone and dilute to 100 ml with methanol. The phenol should be handled carefully; it is toxic and may cause skin irritation.

(b) Dissolve 27.5 g sodium hydroxide, NaOH, in water and dilute to 100 ml.

Mix one part of (a), one part of (b) and two parts of water immediately before use.

Sodium hypochlorite solution, 0.9 g active chlorine in 100 ml.

Variant I: The commercially available sodium hypochlorite must be stored in a dark, cool place. As the active chlorine content changes from time to time, it must be determined every time a new hypochlorite reagent is prepared (weekly).

Dilute 10 ml of sodium hypochlorite solution (usually 8-12 percent active chlorine) to 250 ml with distilled water. Pipette 10 ml of this solution into a titration flask containing 25 ml distilled water, 5 ml concentrated acetic acid and 1 g potassium iodide.

Titrate against 0.1 M sodium thiosulphate (24.82 g $Na_2S_2O_3.5\ H_2O$ in 1 litre of water) with a few drops of starch indicator. Calculate the active chlorine content from the expression:

$$x = \frac{n \cdot a \cdot 35.5 \cdot 25}{b}$$

where

x = mg active chlorine per ml of the hypochlorite solution

n = the exact concentration of sodium thiosulphate in mole/l

a = ml sodium thiosulphate solution consumed in the titration

b = volume, ml, of the hypochlorite solution.

Calculate the volume of sodium hypochlorite solution which contains 0.9 g active chlorine. Add 1 ml of the manganous sulphate solution and dilute to 100 ml. Store the solution in a dark, cook place and prepare it weekly.

Variant II: Dissolve 2.5 g of Trion (common name for 1.3-dichlorcyanuric acid) in ammonia-free water. Add 1 ml of the manganous sulphate solution and dilute to 100 ml. Store the solution in a cold place; a new solution should be prepared each week.

Ammonium stock solution. Dissolve 0.0472 g of ammonium sulphate $(NH_4)_2SO_4$, dried previously in a dessicator, in water and dilute to 100 ml in a volumetric flask. Add one drop of chloroform for preservation. 1 ml contains 0.100 mg of NH_3-N (ammonia nitrogen).

Ammonia standard solution. Pipette 10.00 ml of the stock solution into a 100 ml volumetric flask and dilute to the mark; 1 ml contains 0.010 mg of NH_3-N. The solution is stable only for some hours.

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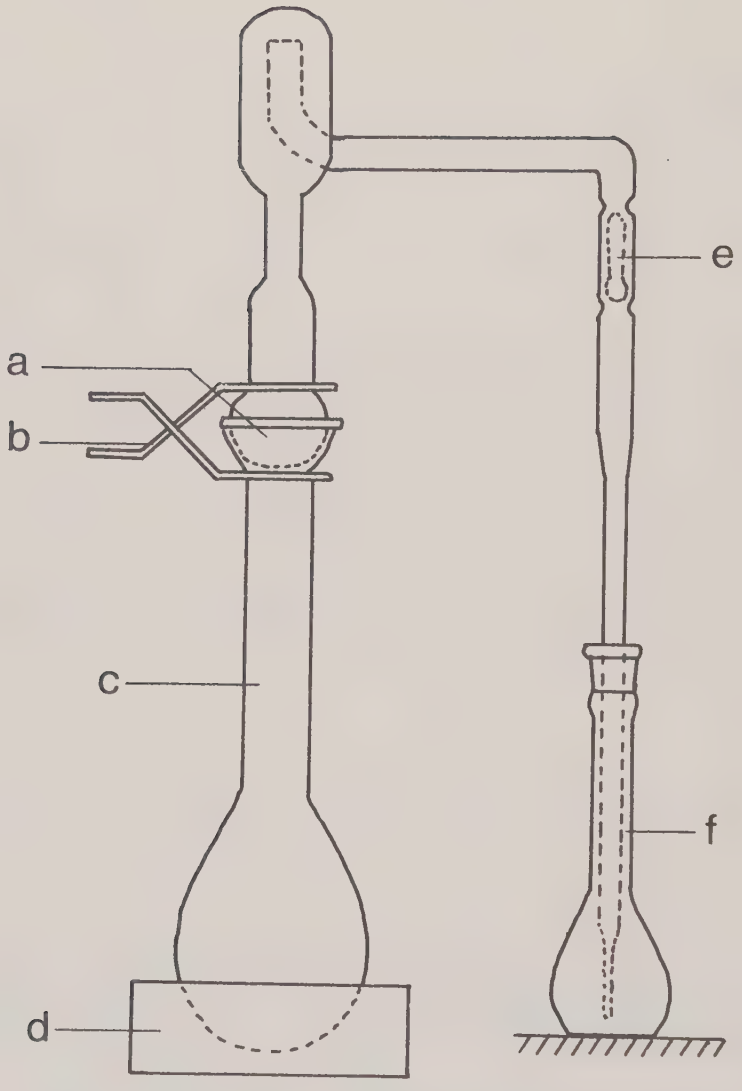

Figure 13. Distillation apparatus

(a) spherical joint 28115
(b) clamp
(c) Kjeldahl flask
(d) heating unit
(e) back pressure valve
(f) measuring flask 25 ml

16.9.2.4 Equipment

- Glass apparatus (Figure 13).

- Volumetric flasks, 25 ml.

- Colorimetric equipment: one of the following is required:

  (a) spectrophotometer, for use at 625 nm with 1 cm photometric cells (or longer as required);
  (b) filter photometer, equipped with a red-orange filter having a maximum transmittance near 625 nm and 1 cm photometric cells (or longer as required).

16.9.2.5 Calibration

Using the ammonia standard solution a series of calibration standard solutions are prepared according to the table instructions given in the preceding method (16.9.1).

The calibration standards are then analysed. In order to control the entire analytical procedure, it is advisable to distill also the standards. If such a calibration is compared with a second one, which has been prepared without distillation, it can be seen that no losses of ammonia occur during the distillation.

The blank which is obtained in the calibration, by adding reagents to the ammonia-free water, compensates for the residual ammonia in that water as well as in the reagents. However, when analysing the water samples only the reagents, and no water, are added. Thus for the analysis a reagent blank is needed, and is obtained in the following way. To three 25 ml portions of ammonia-free water the normal reagent volumes are added, as described below. To another three portions double the amounts of the reagents are added. After the colour development (45 minutes) 4 ml of ammonia-free water is added to each of the samples with single volumes of reagents. The absorbances of all the samples are measured. The blank absorbance is the difference between the mean values from the two sets of samples (see also paragraph 16.9.1.5).

16.9.2.6 Procedure

Distillation. Transfer a suitable sample volume (max. 25 ml), containing not more than 35 µg NH_3-N into the distillation flask with a pipette. If the sample is acidic (certain types of waste waters from industries) or has been preserved with acid, a neutralization must be done. For this purpose, add three drops of the bromothymol blue indicator and sodium hydroxide as required until the colour change of the indicator. The strength of the hydroxide has to be chosen according to the acidity of the sample. The total volume of the sample should not considerably exceed 25 ml after the neutralization.

Use a 25 ml volumetric flask as a trap, containing 1 ml of 0.1 M sulphuric acid. The amount of sulphuric acid must be kept under 0.12×10^{-3} moles if the colorimetric determination is performed directly in the volumetric flask and the given reagent amounts are used. If the sample volume in the distillation flask is less than 25 ml, add distilled water up to that volume and a boiling chip. Add 2 ml of the phosphate buffer solution to the sample. While the sample is acidic the ammonia is kept in the solution, but as soon as the sample turns alkaline, loss of ammonia can occur. Carry out this step carefully and as fast as possible.

Assemble the distillation apparatus. The tip of the delivery capillary should be submerged in the trap (volumetric flask). Shake carefully the entire assembly. Place the distillation flask on the pre-heated heating block. Continue the distillation for another three minutes after steam has been formed. It is possible to follow the steam flow through the system, and to hear it leaving the capillary tip of the delivery tube with a rattling noise. Cool the trap during the distillation. Remove the trap, before the heating is turned off, in order to avoid back flow. Cool the trap to room temperature.

NH_3-N 10/NO_2-N 1

Colorimetric determination. Transfer 2 ml phenate reagent and 2 ml hypochlorite solution with pipettes to the volumetric flask (trap) containing the acidic distillate. Mix thoroughly after every addition. Dilute to 25 ml with water.

Measure the colour after 45 minutes against distilled water, at 625 nm in a cell of 1 cm light path. The colour is stable for up to 24 hours. From time to time a reagent blank is run together with the samples for control of the reagents.

16.9.2.7 Calculation

The absorbance of each sample is corrected for the absorbance due to the reagent blank. The corrected absorbance and the calibration graph are then used for evaluation of the concentration of ammonia nitrogen in the sample. The result obtained is correct only if the sample was not diluted prior to the analysis. Otherwise the concentration of ammonia nitrogen is calculated according to the formula:

$$x = \frac{a \cdot 25}{c}$$

where:

x = concentration of ammonia nitrogen, NH_3-N, in µg/l in the sample

a = concentration of ammonia nitrogen in µg/l as found from the calibration graph

c = sample volume, ml, used for the analysis.

If the result is wanted as µg/l of ammonia, NH_3, the value obtained above is multiplied by 1.214.

16.10 Determination of nitrite

16.10.1 Introduction

This method is applicable for the determination of nitrite nitrogen (NO_2-N) in most types of waters (including sea water and waste water). It constitutes a modification of the well known Bendschneider and Robinson method(Koroleff, 1973).

The determination of nitrite is based on the classical Griess's reaction, in which the nitrite ion at pH 1.5-2.0 is diazotized with sulphanilamide, resulting in a diazo compound, which in turn is coupled with N-(1-naphthyl)-ethylenediamine to form a highly coloured azo dye with an absorption maxima at 545 nm that is measured colorimetrically.

The colorimetric reaction is almost specific for nitrite ions. Interferences may be caused by aromatic amines, copper (more than 0.5 mg/l), iodide ion (more than 0.1 mg/l). Suspended matter and strong colour in the sample interfere, too.

This method is not appreciably affected by salinity, small changes in reagent concentration or volume, or by temperature. Of the interfering compounds, none is normally present in significant amounts in the ocean, inshore or estuarine waters unless excessive pollution from land drainage is encountered.

16.10.2 Sample storage and preparation

Nitrite is an intermediate compound in the simplified redox chain ammonia - nitrite - nitrate. Samples for analysis of nitrite cannot be properly preserved. Storage in a dark and cold place can be tolerated for up to about five hours. If possible, samples should be analysed without delay.

Avoid filtering the samples, and use coprecipitation as pretreatment: Add 0.2 ml magnesium sulphate solution and 0.2 ml 4 M sodium hydroxide solution per 100 ml sample; agitate, and after 30 minutes, take the decanted part of the sample for analysis.

Samples that are slightly turbid and contain no other disturbing substances (certain fresh waters, sea water from near-shore areas) may be analysed together with turbidity blanks instead of being pretreated.

16.10.3 Reagents

Magnesium sulphate solution, about 2 M. Dissolve 50 g $MgSO_4.7H_2O$ in water and dilute to 100 ml.

Sodium hydroxide solution, 4 M. Dissolve 16 g NaOH in water and dilute to 100 ml.

Sulphanilamide reagent. Dissolve 8 g sulphanilamide, $NH_2C_6H_4SO_2NH_2$, in a mixture of 80 ml concentrated hydrochloric acid (HCl, 37 percent sp. gr. 1.19) and 400 ml water. Dilute to 500 ml. The reagent is stable for several months.

Diamine solution, N-(1-naphthyl)-ethylenediamine dihydrochloride. Dissolve 0.8 g of $C_{10}H_7NHCH_2CH_2NH_2.2HCl$ in water and dilute to 500 ml. Store the solution in a dark bottle. Renew the solution about once a month, or as soon as it develops a strong brown colour.

Nitrite stock solution. Anhydrous sodium nitrite, $NaNO_2$ p.a. is dried at 110°C for some hours. Dissolve 0.3449 g of the dry salt in water and dilute to 1 000 ml. Store the solution in a dark bottle with 1 ml chloroform as preservant. The solution is stable for at least 1-2 months. 1 ml contains 5 µgat or 70 µg nitrogen as NO_2-N.

Nitrite working solution. 10 ml of the stock solution is transferred to a volumetric flask and diluted to 1 000 ml. The solution must be used the same day. 1 ml contains 0.05 µgat or 0.7 µg NO_2-N.

16.10.4 Equipment

- Test tubes or volumetric flasks with a capacity of 25 ml.
- Automatic syringe pipettes or piston pipettes for dispensing of reagents (not necessary, but very handy equipment).
- Colorimetric equipment, one of the following is required:
  (i) Spectrophotometer for use at 545 nm, with 1 cm cells and longer as required
  (ii) Filter photometer, equipped with a yellow-green filter having maximum transmittance near 545 nm. 1 cm cells or longer as required.

16.10.5 Calibration

Depending on the type of the samples to be analysed, either one of the two tables with calibration series should be used.

For sea water samples. Using the nitrite working solution (1 ml = 0.05 µgat) a series of standard solutions is prepared by diluting with distilled water. If 100-ml measuring flasks are used, the table below can be followed.

0.10	ml	working	solution/100	ml	=	0.05	µgat/l	NO_2-N
0.20	"	"	"	"	=	0.10	"	"
0.50	"	"	"	"	=	0.25	"	"
1.00	"	"	"	"	=	0.50	"	"
2.00	"	"	"	"	=	1.00	"	"

From the flasks, 25 ml samples are transferred to stoppered test tubes or volumetric flasks. Distilled water is used as the blank. The reagents are added and the analysis is performed as described below. The absorbances (corrected for the blank) are plotted versus concentration on a millimetre paper. In a 10 cm cell, the concentration 1 µgat/l NO_2-N gives an absorbance of about 0.500.

NO_2-N 3/NO_3-N 1

For fresh water and waste water. Using the same nitrite working solution (1 ml = 0.7 µg) the following dilutions are prepared:

2	ml	working	solution/	100	ml	=	14 µg/l	NO_2-N
5	"	"	"	"		=	35	" "
10	"	"	"	"		=	70	" "
20	"	"	"	"		=	140	" "
40	"	"	"	"		=	280	" "

These samples are treated and the results plotted as described above.

16.10.6 Procedure

For precise analysis of low concentrations of nitrite in water, any turbidity in the sample must either be removed or compensated for by a turbidity blank.

25 ml of the neutral sample is transferred to a test tube or volumetric flask. If a turbidity blank is to be analysed, another 25 ml is transferred to a second container. Add 0.5 ml of the sulphanilamide solution to the sample and turbidity blank, then mix well. After not less than three minutes, but not longer than eight minutes, 0.5 ml of the diamine solution (reagent) is added to the sample but not to the turbidity blank. Mix the sample once again.

After ten minutes, the absorbancesof the sample and the turbidity blank are measured in a photometer at 545 nm in a cell length as required. The colour intensity is constant for two hours. The concentration of the sample is evaluated from the calibration graph, when the absorbance of the turbidity blank has been subtracted from that of the sample.

As the addition of acid to a water sample usually changes its turbidity, it is important that the acidic sulphanilamide solution is added not only to the sample, but to the turbidity blank as well.

16.11 Determination of nitrate

16.11.1 Introduction

This method based on a previous publication (Koroleff, 1973) is applicable for the determination of nitrate (or rather the sum of nitrate and nitrite) in natural water and sewage water.

Nitrite concentrations up to about 560 µg (40 µgat) of nitrogen (NO_3-N) per litre can be determined without prior dilution of the samples.

The method is based on the following principle:

Nitrate is reduced to nitrite almost quantitatively (about 90-95 percent) by amalgamated cadmium. The nitrite is then determined according to the classical Griess's reaction as described in 16.10.

The reduction is carried out at a pH of about 8.5. An ammonium chloride buffer is added to the sample to control the pH and to complex the liberated cadmium ions.

If the sample contains considerable amounts of nitrite, a separate determination of nitrite must be done and the result subtracted from that obtained with this method.

The colorimetric reaction is almost specific for nitrite ions. Interferences may occur, caused by aromatic amines, copper (more than 0.5 mg/l), and iodide ion (more than 0.1 mg/l). Suspended matter and a strong colour in the sample also interfere. Instead of filtering the sample, use the precipitation technique.

At concentrations higher than about 210 µg (15 µgat) of NO_3-N in sea water, the dissolved salts cause a deviation from the linear relationship between concentration and the intensity of the coloured nitrite complex. However, nitrate concentrations of that order occur in sea water only in heavily polluted areas.

16.11.2 Sample storage and preparation

The analysis should preferably be undertaken immediately after the sampling. If this is not possible, the samples may be preserved by the addition of 1 ml 4 M sulphuric acid per litre.

Suspended matter and a strong colouration in the sample are removed by using a precipitation technique: Add 0.2 ml of magnesium sulphate solution and 0.2 ml of sodium hydroxide solution (4 M) per 100 ml sample. Agitate, and after 30 minutes, take the decanted solution for analysis.

16.11.3 Reagents

Generally, the ordinary distilled or de-ionized water of the laboratory should be suitable for the analysis of nitrite as well as nitrate. However, if problems are encountered with the quality of the water, "nitrogen-free" water has to be prepared.

Distilled (or de-ionized) water is distilled in all-glass equipment. For this distillation 2 ml concentrated sulphuric acid and 1 g potassium peroxodisulphate ($K_2S_2O_8$) are added per litre.

All chemicals should be of reagent grade (pro analysi).

Hydrochloric acid, about 0.5 M. Dilute 10 ml concentrated HCl (sp. gr. 1.19) to 250 ml with water.

Mercuric chloride solution, 1 percent. Dissolve 1 g $HgCl_2$ in 100 ml water.

Concentrated buffer solution. Dissolve 250 g NH_4Cl in 1 litre of water. Dilute buffer solution. Dilute 20 ml of the concentrated buffer to 1 litre with water. Renew daily.

Hydrochloric acid, 2 M. Dilute 166 ml concentrated HCl (sp. gr. 1.19) to 1 litre with water.

Acid washing solution. Dissolve 4 g NH_4Cl in water, add 15 ml 2 M hydrochloric acid and dilute to 1 litre with water.

Sulphanilamide reagent. Dissolve 8 g sulphanilamide, $NH_2C_6H_4SO_2NH_2$, in a mixture of 80 ml concentrated hydrochloric acid (sp. gr. 1.19) and 400 ml water. Dilute to 500 ml with water. The reagent is stable for several months.

N-(1-naphthyl)-ethylenediamine dihydrochloride solution. Dissolve 0.8 g $C_{10}H_7NHCH_2CH_2NH_2.2HCl$ in water and dilute to 500 ml. Store the solution in a dark bottle. Renew the solution about once a month, or immediately if it develops a strong brown colour.

Magnesium sulphate solution, about 2 M. Dissolve 50 g $MgSO_4.7\ H_2O$ in water and dilute to 100 ml.

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Sodium hydroxide solution 4 M. Dissolve 160 g NaOH in water and dilute to 1 litre.

Nitrate stock solution. Dissolve 1.0111 g potassium nitrate, KNO_3, in distilled water and dilute to 1 000 ml. Add 1 ml chloroform as preservant. The solution is stable for several months if evaporation is prevented. 1 ml = 10.0 µgat (140 µg) NO_3-N.

Diluted nitrate solution. 10 ml of the stock solution is diluted with distilled water to 1 000 ml. 1 ml = 0.1 µgat (1.4 µg) NO_3-N.

Synthetic sea water. Dissolve 310 g sodium chloride, NaCl, 100 g magnesium sulphate, $MgSO_4 \cdot 7H_2O$ and 0.50 g sodium hydrogen carbonate, $NaHCO_3 \cdot H_2O$ in 10 litres of distilled water. The salinity of this water is about 33.7 percent. When working with Baltic water, 200 ml of this solution is diluted to 1 000 ml with distilled water.

Amalgamated cadmium. If granulated cadmium (for instance, Merck Cadmium grob gepulvert, zur Füllung von Reduktoren) is not available, one has to prepare it in the following way:

File sticks of pure cadmium metal (reagent grade) with a coarse metal hand file (about second cut) and collect the fraction which passes a sieve with 1-mm openings and is retained on a sieve with 0.5-mm openings[1/]. Calculate the required amount of cadmium about 35 g per column (see Figure 14). Rinse the filings quickly with 0.5 M hydrochloric acid and then with distilled water until the rinse water does not give the chloride reaction with silver nitrate.

Transfer about 35 g of the washed metal into a 100 ml glass bottle and fill the bottle with the mercuric chloride solution so that all air is excluded from the bottle. Seal the bottle with a glass stopper. After this step avoid all contact between air and the metal. Rotate the bottle for 90 minutes in a horizontal position with suitable equipment. Open the bottle and rinse out the turbid sublimate solution with distilled water which is introduced through a glass tube at the bottom of the bottle. Collect the sublimate solution.

Do not pour the used mercuric chloride solution into the sewer. When a suitable amount is collected, add 25 ml concentrated HCl per litre and precipitate with hydrogen sulphide (H_2S). Store the precipitate.

16.11.4 Equipment

- Reduction columns (see Figure 14)
- Volumetric flasks or test tubes with a capacity of 25 ml. The tubes should either be graduated or marked at the 25 ml volume.
- Colorimetric equipment; one of the following is required:
  (a) spectrophotometer, for use at 545 nm and provided with cells of 1 cm (and 5 cm) length, or
  (b) filter photometer, equipped with a filter having maximum transmittance at (or near) 545 nm, with cells of 1 cm (and 5 cm) length.

1/ There are different sieves available commercially. The following table gives the suitable sieves.

Opening:	mm	DIN	Mesh/cm	Mesh/inch	Sieve No.
Upper sieve	1	6	7	17.2	18
Lower sieve	0.5	12	13	32	35

16.11.5 Preparation of the reduction columns

Place a small ball of thin copper wire at the bottom of the reduction column and fill it entirely with water. Open the bottle containing the amalgamated cadmium filings and put a finger (or a rubber plate) on the opening. Turn the bottle obliquely downward and place the opening under the water surface in the reductor column. The metal should flow down into the reductor column without coming into contact with air. Make sure that no cavities are formed in the reductor. Fill the column with the metal to about 1 cm below the reservoir and plug the column with glass wool.

Flush the column a couple of times with the acid washing solution and with the dilute buffer solution until the effluent shows a weakly alkaline reaction (for instance, blue colouration with bromothymol blue). The column should never be left with the acid washing solution. Flush the column weekly when it is used regularly or more often if the flow decreases through the column. A newly prepared column reduces almost immediately 95-100 percent. If the reduction efficiency decreases below 85 percent, empty the column, wash the filings quickly with 2 M HCl and rinse very thoroughly with distilled water. Dry the filings, sieve again, and reamalgamate as described above.

If a column does not give a proper reduction when it is newly prepared it may be "started" by running through a nitrate standard solution. In most cases, less than one litre will suffice.

16.11.6 Calibration

A series of standard concentrations is prepared from the nitrate stock solution and the diluted nitrate solution by diluting with distilled water or synthetic sea water. As there is no salt error at concentrations below about 15 µgat/l (210 µg/l) the synthetic sea water will probably seldom be required.

As several columns are likely to be calibrated at the same time, the standard solutions are prepared in at least 1 litre quantities:

ml diluted nitrate solution/1 000 ml	µgat/l	or	µg/l of NO_3-N
1.00	0.10		1.4
5.00	0.50		7.0
10.00	1.00		14.0
50.00	5.00		70.0

ml nitrate stock solution/1 000 ml	µgat/l	or	µg/l
1.00	10.0		140
2.00	20.0		280
3.00	30.0		420
4.00	40.0		560

The standard solutions are analysed as described below. Duplicates of each solution must be analysed.

The absorbances are plotted versus concentration for each of the reduction columns. If the results are very uniform from column to column, one calibration graph may be used for the subsequent evaluation of the samples. However, most probably one graph will be required for every column.

NO_3-N 5

If samples have been treated according to the precipitation procedure in paragraph 16.11.3, then the calibration solutions and blank samples should be treated in the same way.

16.11.7 Procedure

Reduction of nitrate. Neutralize acid samples with 0.1 or 0.5 M NaOH after addition of two drops of bromothymole blue indicator. (Calculate prospective dilution.)

(1) Fill the reservoir of the reduction column with the dilute buffer solution and let it flow through. Discard the solution.

(2) Fill the reservoir again with 50 ml distilled water and 1 ml buffer solution (250 g NH_4Cl/l). Discard the first 20 ml which flows through the column and collect 25 ml in a volumetric flask. Proceed in the same manner with all the columns in order to obtain a blank value. These blank samples are then analysed for nitrite as described in (9) and onward. If samples have been treated according to the precipitation procedure in paragraph 16.11.3, then the blank samples and the calibration solutions should be treated in the same way.

(3) The reduction efficiency of each column must be controlled for every analytical batch. The standard concentration used for this control depends on the types and concentration range of the samples. Analyse duplicates of this control standard as described in (4) to (10).

(4) Take a 50 ml sample or less (containing 528 µg or 2 µgat NO_3-N) and adjust the volume to 50 ml.

(5) Add 1 ml of buffer solution (250 g NH_4Cl/l) and mix; use an automatic pipette if possible.

(6) Draw off the excess solution in the reductor by using a capillary-tipped glass tube connected to a suction pump and proceed immediately with (7).

(7) Transfer the whole sample (4) to the reservoir of the reductor. Discard the first 10 ml flowing through the column and use 10 ml to rinse a 25 ml volumetric flask (or test tube). Then collect 25 ml in the flask or tube. 50 ml should flow through the column in ten to twelve minutes. Proceed as described in (9). (This determination of nitrite should be carried out within one hour.)

(8) Draw off the remaining sample from the reductor and begin directly with the next sample.

The reductor column must be washed immediately after every analytical batch. Fill the reservoirs twice with the dilute buffer solution. Cover the reductors and make sure that they do not dry.

Determination of nitrite

(9) To 25 ml of the reduced sample, coming from the reductor, add to samples, standard solutions and blank samples 0.5 ml sulphanilamide solution and mix. After not less than 3 minutes, and not longer than 8 minutes, add 0.5 ml of N-(1-naphthyl)-ethylenediamine solution and mix again.

(10) The absorbance of the coloured sample is measured after 10 minutes, but within two hours, against the reference sample at 545 nm. Use a cell of suitable length depending on the intensity of the colour. (The absorbance of the blank sample against water should not exceed 0.010, measured in a 1 cm cell.)

16.11.8 Calculation

Subtract the absorbance of the blank sample from the absorbances of the samples and evaluate their concentrations from the calibration graph.

The result obtained is the sum of the concentrations of nitrite and nitrate nitrogen in each sample. If a sample contains a significant concentration of nitrite, this must be compensated for by subtracting the result found from a separate determination according to the previous method (16.10).

If the sample was diluted prior to the reduction this must be taken into account in the calculation:

$$\mu gat/l \text{ (or } \mu g/l) \ NO_3\text{-N} = \frac{A \times B}{V}$$

where:

A = nitrate concentration (μgat/l or μg/l) as obtained from the calibration graph

V = ml of original sample before dilution

B = final volume, after dilution.

For example: A 25 ml sample was diluted to 100 ml and of this 50 ml was analysed. The result obtained from the calibration graph was 430 μg/l

Then $\frac{430 \times 100}{25} = 1\ 720\ \mu g/l\ NO_3\text{-N}$

From the results of the analysed standards and samples, done at least in duplicates, one has to determine with what accuracy the nitrate concentrations should be reported. As the behaviour of the reduction columns is somewhat "personal", the accuracy and precision obtained in routine work at different laboratories varies. However a detection limit lower than 0.1 μgat/l or 1.4 μg/l can hardly be expected. Thus the figures have to be stated accordingly.

To check the reduction efficiency of the reductor (4) a nitrate standard solution of suitable concentration is analysed. For instance, a solution of 1 μgat/l (14 μg/l) is analysed and an absorbance of 0.241 is obtained when using a 5 cm cell. An analysis of the nitrite solution with the same concentration gives the absorbance 0.258 under the same conditions. The reduction efficiency is then: $\frac{0.241 \times 100}{0.258} = 93$ percent.

16.12 Determination of total nitrogen

16.12.1 Introduction

The method is designed for application to natural water (including sea water) and waste water.

It is based on the following principle:

Organic and inorganic nitrogenous compounds are oxidized under pressure, by potassium persulphate in alkaline solution to nitrate. The nitrate is reduced to nitrite and determined colorimetrically as described in 16.10.

Nitrogen gas, dissolved in the water sample will not be oxidized to nitrate but most substances containing organic nitrogen will be determined quantitatively.

tot N 2

16.12.2 Sample storage

The samples should be stored in small, acid-washed glass bottles with ground glass stoppers. They should be analysed directly after the sampling. Samples in which the total nitrogen content is to be determined should not be preserved.

16.12.3 Reagents

Unless indicated, all chemicals should be of reagent grade (pro analysi). Use only those qualities which contain as little of nitrogen compounds as possible.

All water used in the analysis should be freshly prepared and "nitrogen-free" (see 16.9.1.3).

Sodium hydroxide solution, 0.15 M. Dissolve 6 g NaOH in water and dilute to 1 litre.

Oxidation reagent. Dissolve 5 g potassium persulphate, $K_2S_2O_8$ (the only suitable product is Merck No. 5092, which is N-free), in 0.15 M sodium hydroxide and dilute to 500 ml with the same solution.

Store the solution in a dark glass bottle, which should always be kept well closed. The solution is stable for several weeks.

Sulphuric acid, 1.25 M. Add carefully 70 ml concentrated H_2SO_4 to 500 ml water. Cool and dilute to 1 litre.

Bromothymol blue, 0.05 percent. Dissolve 50 mg bromothymol blue in 20 ml ethanol and dilute to 100 ml with distilled water.

16.12.4 Equipment

- Reduction columns (see Figure 14).
- Volumetric flasks or test tubes with a capacity of 25 ml. The tubes should either be graduated or marked at the 25 ml volume.
- Colorimetric equipment: one of the following is required:
  (a) spectrophotometer, for use at 545 nm and provided with cells of 1 cm (and 5 cm) length, or
  (b) filter photometer, equipped with a filter having maximum transmittance at (or near) 545 nm, with cells of 1 cm (and 5 cm) length.
- Oxidation bottles, 50 ml. One of the following types may be used:
  (a) 50 ml Jena "Rollrandflasche mit Bügelverschluss" No. 4176b (Figure 12). The original gasket in the stopper must be replaced by one made of colourless, silicone rubber. This can be cut from a tube with 16 mm outer diameter and 10 mm inner diameter.
  (b) 50 ml Pyrex or Jena bottles (ordinary or Erlenmeyer shape) with screw caps of polypropylene. Sovirel bottles may be used (No. 262-33) if the stopper is replaced by one of polypropylene (Nalgene No. 2150, size 28).
  (c) 50 ml Pyrex or Jena glass bottles with ground glass stoppers and metal clamps.
  (d) 100-150 ml Erlenmeyer flasks of alkali resistent glass. The use of these open flasks has disadvantages in the oxidation process, but this fact must be accepted if a pressure cooker or autoclave is not available.

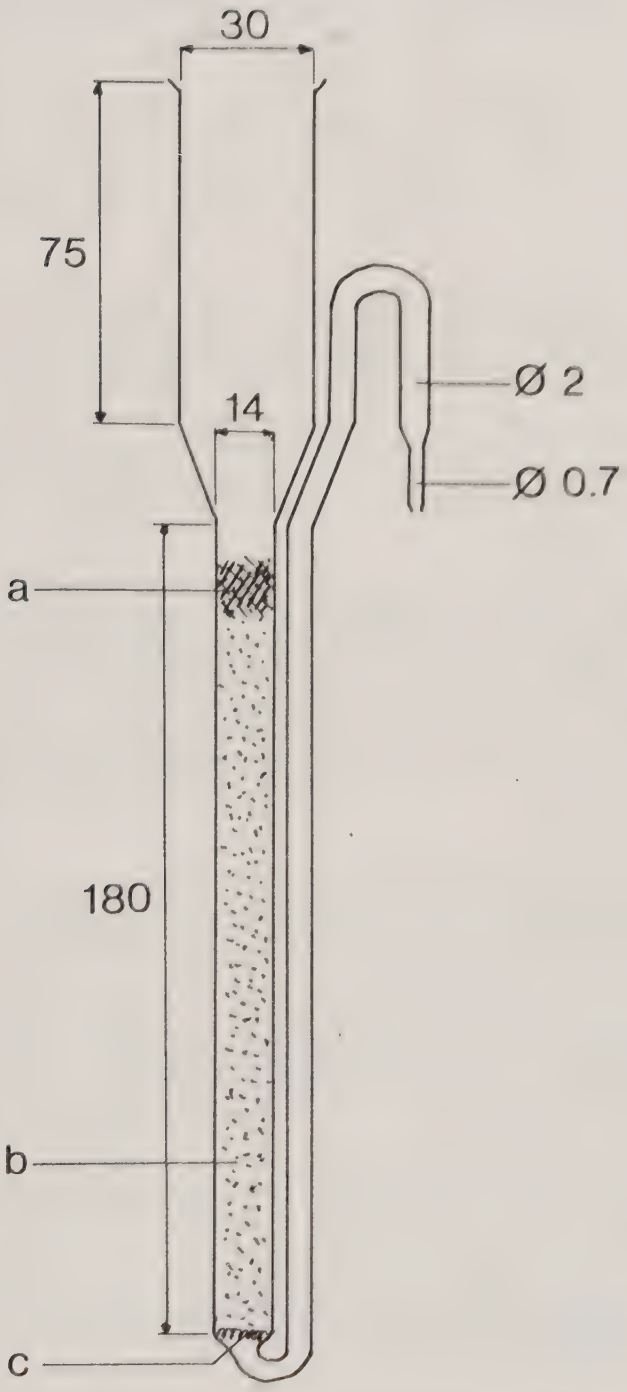

Figure 14. Reduction column for analysis of nitrate and total nitrogen (all measures given in millimetres)

(a) glass wool
(b) Cd-amalgam
(c) copper wire

tot N 4

Regardless of the type of the oxidation containers used they must not only be washed carefully prior to use, but they should also be treated with dichromate sulphuric acid and rinsed with distilled water. When not in use, they should be stored closed (covered) so that they do not collect dust and ammonia vapour.

- Autoclave, or an ordinary household pressure cooker.

- 50-ml volumetric flasks, or 100-150 ml Erlenmeyer flasks and 50-ml measuring cylinders.

16.12.5 Calibration

It has been well documented from experiments, that ammonia, EDTA, urea, pyridine, amino acids, etc., are quantitatively oxidized to nitrate with the given procedure. Thus it is commonly regarded as unnecessary to calibrate this determination of total nitrogen separately, and to rely solely on the nitrate calibration of the reduction columns, as described in 16.11.

However, to check that the entire procedure operates successfully, it may be advisable to oxidize a standard sample and determine the nitrate yield. In this way, the sources of error (leakages from the oxidation bottles or spattering from the flasks, errors in the pH-adjustment, etc.) may be traced.

If a calibration is desired, an amino acid such as glycocoll, amino acetic acid (NH_2CH_2COOH) may be used. Dry the crystals in an oven at 105° C and prepare a solution of suitable concentration by weighing accurately a portion of the substance that is then dissolved in nitrogen-free water. Prepare a series of standard solutions and analyse them according to the procedure described below. Note that the blank sample needed in the calibration compensates for the nitrogen content in the distilled water as well as in the oxidation reagent. Thus 15 ml of distilled water is analysed, using the same procedure. This is in contrast to the blank sample for the analysis of the water samples.

16.12.6 Procedure

Boiling at pressure in closed bottles. Pipette 15 ml of the neutral sample into an oxidation bottle, which has previously been rinsed with acid and then water. If the sample volume is less than 15 ml, dilute to this volume with distilled water.

Add 10 ml of the oxidation reagent, seal and mix. Autoclave the sample for 30 minutes at 125° C. Open the oxidation bottle, while still hot, <100° C. Add 0.5 ml 1.25 M H_2SO_4. Mix well during the cooling period. (If the sample is sea water, the Mg-Ca-precipitate will now dissolve.)

Transfer the cooled sample to a 50 ml volumetric flask. Rinse the bottle and transfer also the rinsing water to the volumetric flask. Add one drop of bromothymol blue indicator and neutralize the sample using the 0.15 M sodium hydroxide. Dilute to 50 ml and proceed from (5) in the nitrate determination (16.11.8).

Boiling in open flasks. As was pointed out above, this method has disadvantages because of the risk of spattering from the flask during the boiling.

Pipette 15 ml of the neutral sample into an Erlenmeyer flask, which has previously been rinsed with acid and then water. If the sample volume is less than 15 ml, adjust to this volume with distilled water.

Add 10 ml of the oxidation reagent and mix. The mixture is then gently boiled in a small beaking manil (preferably not a hot plate, because of the increased risk of spattering) for 20-30 minutes or until the volume is reduced to about 10 ml. Add 0.5 ml of 1.25 M H_2SO_4 and mix well. (In a sea water sample the precipitates of Ca-Mg-hydroxide will now dissolve.)

Add one drop of bromothymol blue indicator and neutralize the sample using the 0.15 M sodium hydroxide. Dilute to 50 ml with distilled water in a measuring cylinder and proceed from (5) in the nitrate determination (16.11.8).

Analysis of the blank sample. Despite all precautions, the oxidation reagent may absorb ammonia. It is therefore necessary to analyse daily a blank sample of the oxidation reagent. This analysis should preferably be carried out in duplicate. Analyse 10 ml portions of the oxidation reagent according to either of the chosen procedures. Use the mean value of the blank samples for correction of the sample absorbances.

16.12.7 Calculation

The result is calculated as described in the nitrate determination method (16.11). Correct the absorbance of the sample for that of the blank, as above.

If no separate calibration has been performed for this determination of total nitrogen, the graph for the nitrate determination is used. In this case, however, it must be remembered that the nitrate graph is based on the analysis of a 50 ml sample, whereas the total nitrogen sample is diluted 3.33 times (15 ml diluted to 50 ml). This dilution factor must, of course, be taken into account.

A general statement on the accuracy and precision of the method cannot be made. In addition to the determination of nitrate, this procedure involves several critical steps, such as the risk of contamination of the oxidation reagent, possible losses during the oxidation, the pH-adjustment, etc. An accuracy better than $\pm$ 10 percent can probably not be expected in routine work.

16.13 Determination of Kjeldahl nitrogen

16.13.1 Introduction

This method is applicable for the determination of Kjeldahl nitrogen in natural water (including sea water) and waste water. Kjeldahl nitrogen includes ammonia and organic nitrogen but does not include nitrate and nitrite nitrogen.

The method is based on the following principle:

In the presence of sulphuric acid, potassium sulphate and copper sulphate catalysts, organic nitrogen is converted by digestion to ammonia nitrogen, which is distilled from the alkalized sample and absorbed in an acid media. The ammonia is determined colorimetrically by the indophenol method (16.9.2).

16.13.2 Sample storage

The most reliable results are obtained on fresh samples. If prompt determination is not possible (that is directly after sampling) store the samples at 4 °C, for a maximum of three days or preserve the samples by addition of 1 ml of 2 M sulphuric acid per litre.

16.13.3 Reagents

All water used for dissolving reagents and dilution of samples must be distilled (see 16.9.1.3). All chemicals should be of reagent grade (pro analysi).

Sulphuric acid, H_2SO_4. Concentrated.

Potassium sulphate, K_2SO_4.

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Copper sulphate solution. Dissolve 25 g copper sulphate, $CuSO_4 \cdot 5H_2O$, in distilled water and dilute to 1 litre.

Sodium hydroxide solution 10 M. Dissolve 400 g sodium hydroxide, NaOH, in water, cool and dilute to 1 litre.

Other reagents and solutions according to the second method for the determination of ammonia nitrogen (16.9.2).

16.13.4 Equipment

- Digestion apparatus. Consists of a number of Kjeldahl flasks (100 ml) with ground spherical joints, individual heating possibility (electrical or flame) and well ventilated hood (Figure 13).

- Volumetric flasks, 25 ml.

- Colorimetric equipment: one of the following is required:
  (a) spectrophotometer, for use at 625 nm with 1 cm photometric cells (or longer as required)
  (b) filter photometer, equipped with a red-orange filter having a maximum transmittance near 625 nm and 1 cm photometric cells (or longer as required).

16.13.5 Procedure

The analysis of nitrogen is very sensitive to air pollution, especially if ammonia is present in the laboratory environment. As the determination is designed for tracing minute amounts of nitrogen, special care must be taken when performing the determination. No smoking is allowed in the laboratory during the ammonia or Kjeldahl determination; also remove bottles and containers with ammonium compounds from the vicinity.

Digestion. Place a suitable sample volume in a 100 ml Kjeldahl flask. Suitable sample volume is 25 ml. The ammonia content of the volume should not exceed 40 µg (2.9 µgat). Add 4 ml concentrated sulphuric acid, 1 ml copper sulphate solution and 6 g potassium sulphate. Place the Kjeldahl flask in the apparatus or on the heating plate in the hood. Heat the contents gently and avoid loss through foaming. When the water has boiled off from the sample, the sulphuric acid is present and the sample will turn dark because the sulphuric acid decomposes the organic matter into elementary carbon, which will be oxidized further to carbon dioxide.

As the digestion proceeds, the original dark colour fades to light green. Continue the heating for an additional 30 minutes after the solution has become light green. Allow flask and contents to cool and add then very carefully about 25 ml water. Let the sample cool once again, prior to distillation. Perform the determination simultaneously on an acid blank.

Distillation. Perform the distillation according to the ammonia method (16.9.2) except that 15 ml 10 M sodium hydroxide solution should be used instead of 1 ml 0.5 M NaOH to neutralize the digested sample. Use a pipette filler when measuring the 10 M NaOH. Alkali of this concentration is harmful to the mouth and skin.

Colorimetric determination. Follow instructions in the method for ammonia (16.9.2).

16.13.6 Calculation

For the evaluation of the absorbances of the samples, the graph in the ammonia method (16.9.2) is used. However, it must be remembered that this graph is based on the analysis of a 25 ml sample. If a smaller volume has been used in this analysis, the dilution factor (25 divided by the actual sample volume) must be taken into account in the calculation. Give the result as Kjeldahl nitrogen µg/l (or mg/l or µgat/l) of NH_3-N (ammonia nitrogen). If the result is wanted as ammonia instead of ammonia nitrogen, the NH_3-N figure is multiplied by 1.213.

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Information has been taken from the New Baltic Manual (Carlberg, 1972) for para. 2, 4, 5, 6 and 7 and from the NORFORSK publication 1973:3 (Scandinavian Council for Applied Research) for para. 10, 11 and 12.

17. METHODS FOR DETERMINATION OF SOME SELECTED ORGANIC PARAMETERS IN WATER

17.1 Permanganate value (oxidability) of organic matter in natural waters

17.1.1 Introduction

In investigations of natural fresh waters the permanganate value (frequently called oxidability or chemical oxygen demand) is often estimated. If the investigation concerns a river, analyses of estuarine and even marine samples are often required. However, the methods generally used (oxidation with permanganate or dichromate) are not directly applicable to sea water because of its high content of chloride ions. For use in sea water the permanganate method may be modified by using alkaline oxidation instead of acidic. In the dichromate method, a correction for the chloride content can be made in the calculation, if the concentration of chloride ions in the sample is known.

Since the carbon and hydrogen, but not the nitrogen, in organic matter is oxidized by chemical oxidants, the oxygen consumed in these oxidations is sometimes erroneously considered as indicating the amount of carbonaceous organic matter present. These determinations, however, indicate only the presence of a part of the organic matter, the proportion depending on the chemical oxidant used, the structure of the organic compounds and the analytical conditions. The analysis does not differentiate unstable from stable organic matter. Hence the results obtained are not directly correlated to the amount of oxygen consumed by biological oxidation.

A modification of the permanganate method, generally used in fresh water investigations in Sweden, is given below.

To a measured volume of the sample a known amount of the oxidant and sodium hydroxide is added; the sample is then heated in a boiling water bath. After 20 min the oxidation is interrupted by cooling and the remaining amount of oxidant is, after acidification, determined iodometrically.

Fresh water samples (all samples containing less than 300 mg/l of chloride ions) are oxidized in acid solution instead of the alkaline. However, it should be remembered that the organic substances are not oxidized to the same degree by the acid and the alkaline procedures, the acid oxidation generally being more powerful.

17.1.2 Sampling and storage

The samples are collected with the usual water samples and filled up in bottles of glass or plastic. If not analysed at once the samples may be stored in a refrigerator for about 24 h. Tests have been made by Carlberg concerning the preservation of samples. Deep freezing ($-20^{\circ}C$) and addition of mercuric chloride ($HgCl_2$, saturated solution, 1.5 ml/l sample) were used and samples from all periods of the year were stored for 9–31 days. The results thus obtained were compared with the analyses of the corresponding fresh samples. The results were correct to within $\pm$ 1 mg/l $KMnO_4$ for 51 percent of the samples or to within $\pm$ 3 mg/l for 95 percent of the samples concerning the mercuric chloride preservation. In the same way, the results were correct to within $\pm$ 1 mg/l (39 percent) or $\pm$ 3 mg/l (92 percent) for the frozen samples. As the regional variations of the values often are of about the same order, these preservation methods do not seem to be sufficient.

17.1.3 Reagents

Potassium permanganate solution. From an ampoule a 0.1 N solution is made. Alternatively, dissolve 3.16 g $KMnO_4$ p.a. in distilled water and dilute to 1 000 ml; in this case the solution ought to be filtered before storage. Store in a dark glass bottle.

Potassium permanganate working solution, 0.01 N. 100 ml of the stock solution is diluted to 1 000 ml with distilled water.

Sulphuric acid (1+3) solution. One volume of concentrated sulphuric acid, H_2SO_4 p.a., is poured, under constant mixing and cooling, into three volumes of distilled water.

Sodium hydroxide solution, 5 percent. Dissolve 50 g NaOH p.a. in about 50 ml distilled water and then dilute to 1 litre.

Potassium iodide solution, 0.1 M. Dissolve 16.6 g KI p.a. in distilled water and dilute to 1 litre.

Sodium thiosulphate solution, 0.02 N. This is prepared and standardized as for oxygen analysis.

Starch solution or Thyodene indicator. Use the same as in oxygen determination.

17.1.4 Equipment

- Water bath with test tubes, for instance a big kettle equipped with a stand for nine test tubes 37 x 200 mm. These tubes hold 100 ml sample plus reagents. The tubes should be covered with watch glasses during the boiling.

- Heating plate with high effect. Should be capable of getting the hot water bath to boil in about 2 min after the samples are inserted.

- Burette, the same as for oxygen determinations.

17.1.5 Procedure

New test tubes must be oxidized with permanganate after washing. Fill the tubes with distilled water, 5 ml of sodium hydroxide and 10–20 ml 0.1 N permanganate and heat in the water bath. A precipitation of MnO_2 will form. Continue the treatment until no such precipitation is formed.

Heat the water bath to boiling. One hundred ml of the sample is poured into a test tube. Add 5 ml of the sodium hydroxide solution and 20 ml of the 0.01 N permanganate. Mix well and place the tube in the stand. As a blank use 100 ml distilled water treated in the same way (not de-ionized water, because this contains traces of organic material).

When all the samples have been treated in this way the stand is placed in the boiling water bath. The tubes should be covered with watch glasses in order to avoid contamination from the water in the bath. The water bath should be boiling within about 2 min after the samples have been placed in it. Make sure that the water in the bath does not spray into the samples. After heating for 20 min in the boiling water, the stand is transferred to cold water to interrupt the oxidation.

The remaining amount of permanganate is determined by titration. To the cold sample 5 ml of the sulphuric acid (1+3), and 10 ml of the potassium iodide solution are added. The precipitate of alkali metals dissolves and the sample turns yellow and looks like a sample for oxygen determination. The titration is performed as for oxygen with thiosulphate and starch indicator. Titrate to a pale straw colour, add the indicator and proceed with the titration at moderate speed until the sample is completely colourless.

Samples containing less than 300 mg/l of chloride ions are oxidized in the following way: 20 ml of the permanganate solution and 5 ml of the sulphuric acid (1+3) are added. Then after boiling and cooling no further addition of acid is required before the titration.

17.1.6 Calculation

The consumption of potassium permanganate in mg/l in the sample is:

$$KMnO_4 \text{ mg/l} = 3.16 \cdot N \cdot 100 \ (b-s)$$

where

N = normality of thiosulphate solution

b = ml thiosulphate solution consumed by the blank

s = ml thiosulphate solution consumed by the sample

Give two significant figures in the answer.

If the anser is wanted in mg/l oxygen consumed:

$$O \text{ mg/l} = 0.8 \cdot N \cdot 100 \ (b-s)$$

17.2 Biochemical oxygen demand of waste water - Dilution method

17.2.1 Introduction

This method is designed for application to effluents containing no other toxic or interfering substances than active chlorine, sulphur dioxide, hydrogen sulphide or other volatile sulphides.

The biochemical oxygen demand of an industrial effluent is the amount of dissolved oxygen that is consumed in biochemical oxidation of organic matter under specified conditions.

The purpose of a determination of biochemical oxygen demand is to measure the amount of organic material decomposed and oxidized by natural aquatic micro-organisms (mainly bacteria). However, as a direct measurement of the decomposed and oxidized material cannot be made, an indirect measurement via the oxygen consumption has to be done. Under the conditions of the test, however, several inorganic compounds or ions, for instance SO_3^{2-} and Fe^{3+}, may consume oxygen; these interfering substances are therefore removed before the test is started.

The principle of the method is as follows: The pre-treated and suitably diluted sample is seeded with domestic sewage to initiate biochemical oxidation. Inorganic nutrient salts are added and the oxygen content is measured immediately and - in a separate sample - after an incubation period of 7 days at 20°C.

The oxygen dissolved in the sample is determined by adding Mn^{2+} ions, which are oxidized to manganese (III) hydroxide, MnO(OH). On the addition of potassium iodide and acidification an equivalent amount of iodine is liberated and this is titrated with sodium thiosulphate.

In many countries an incubation period of 5 days is commonly applied. The period of 7 days specified in this method is convenient for laboratories working a 5-day week.

17.2.2 Reagents

All chemicals should be of reagent grade (pro analisi). Check the quality of all the distilled water used in this method.

Distilled water sometimes consumes oxygen. Check the quality of the water in this respect by determining the permanganate number of the water directly and after it has been boiled for 20 minutes. The method is described in 17.1. If the difference in the consumption of 0.02 N sodium thiosulphate solution exceeds 0.08 ml, the water should not be used for preparing solutions or as dilution water.

BOD dil 2

Nutrient solutions:

Phosphate buffer. Dissolve separately in sterile, distilled water, 8.5 g potassium dihydrogen phosphate, KH_2PO_4, 21.8 g dipotassium hydrogen phosphate, K_2HPO_4, 33.4 g disodium hydrogen phosphate, $Na_2HPO_4 \cdot 7H_2O$, and 3.24 g potassium nitrate, KNO_3. Mix the solutions and dilute to 1 l with sterile, distilled water. Store in a dark, cool place. The solution easily becomes infected and has a short shelf life. Discard solutions that have become turbid or that are more than one month old.

Because this nutrient solution is susceptible to bacterial contamination, sterilize the water used for its preparation by boiling for a new minutes; sterilize also the bottle for storing the solution by heating it to 120°C for 2 hours.

Do not dip unsterilized objects such as pipettes into the solution. Do not return unused portions to the storage bottle.

Magnesium sulphate solution. Dissolve 22.5 g magnesium sulphate, $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 litre.

Iron (III) chloride and calcium chloride solution. Dissolve separately 0.25 g iron (III) chloride, $FeCl_3 \cdot 6H_2O$ and 27.5 g calcium chloride, $CaCl_2$ in distilled water. Mix the solutions and dilute to 1 litre.

Seed. Domestic sewage, preferably taken from the effluent, from the primary clarifier in a sewage treatment plant. Before use, leave the seed in contact with air in an open vessel for 24 to 36 h at 20°C. Take a portion from the centre of the liquid with a pipette and filter through surgical cotton or a paper filter.

To avoid infection of personnel, extreme care should be exercised when handling the seed. Rinse pipettes and other glassware thoroughly after each test. Do not place used pipettes on laboratory benches, but use a pipette stand. After working with the seed disinfect the hands. At the end of each working day sterilize all the glassware (except the incubation bottles).

Dilution water. Distilled water, free from copper and having an oxygen content, at 20°C, of 8 to 10 mg/l, obtained by bubbling air through the water. If compressed air is used for the purpose it should be purified by filtering. It must not contain foreign substances, suoh as oil, water or exhaust gases. Check the oxygen content as described in paragraph 17.2.6.

Sulphuric acid, 0.05 M. Add 2.8 ml of concentrated H_2SO_4 (sp.gr.=1.84) to about 700 ml of distilled water and dilute to 1 litre.

Sodium hydroxide solution, 0.1 M. Four g NaOH per litre.

Sodium sulphite solution, about 0.0125 M. Dissolve 1.6 g Na_2SO_3 in distilled water and dilute to 1 litre. As this solution is unstable, use only freshly prepared solutions.

Potassium iodide solution. Dissolve 10 g potassium iodide, KI, in distilled water and dilute to 100 millilitres.

Acetic acid, 8.7 M. Add 250 ml of glacial CH_3COOH (sp.gr.=1.049) to 250 ml distilled water.

Starch indicator solution. Dissolve 1 g of "soluble starch" in 200 ml of hot distilled water. Add a small amount (a few mg) of mercury (II) iodide, HgI_2, as a preservative.

Alkaline iodide solution. Dissolve separately 500 g sodium hydroxide, NaOH, and 150 g potassium iodide, KI in distilled water. Mix the solutions and dilute to 1 litre. Add a solution of 10 g sodium azide, NaN_3 in 40 ml of distilled water.

Manganese (II) sulphate solution. Dissolve 480 g $MnSO_4 \cdot 4H_2O$ in distilled water and dilute to 1 litre. Filter the solution if it is turbid.

As alternative to these alkaline iodide and manganese sulphate solutions, the corresponding solutions for determination of dissolved oxygen (para. 16.4.2) may be used.

Phosphoric acid. H_3PO_4, concentrated (sp.gr.=1.83).

Sodium thiosulphate solution, 0.1 N. Dissolve 24.8 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water and dilute to 1 litre. Shelf life limited.

Sodium thiosulphate solution, 0.01 N, standardized and the normality known to 0.0001 N. This solution is prepared by careful dilution of the 0.1 N solution above. Standardization is carried out exactly as described for the somewhat stronger solution used in determination of dissolved oxygen (para. 16.4.2).

Glucose-glutamine solution. Dissolve separately in distilled water 150 mg glucose, $C_6H_{12}O_6$, and 187.2 mg glutamic acid hydrochloride, $C_5H_9NO_4 \cdot HCl$ (both substances dried over phosphorus pentoxide). Mix the solutions in a 1-l volumetric flask and dilute to the mark. The solution should be freshly prepared.

Iodine solution, 0.01 N. Dissolve 1.28 g iodine, I, and 20 g potassium iodide, KI, in 40 ml distilled water in a 1-l volumetric flask and dilute to the mark. Let the solution stand for 24 h before use and store it in a dark, glass-stoppered bottle. Commercially available standard solutions of iodine may be used.

17.2.3 Equipment

- Glass incubation bottles of around 130 ml, with ground-in tapered stoppers. The volume of the bottles with the stopper inserted should be known to the nearest millilitre. This is the same as the Winkler bottle in the determination of dissolved oxygen.

- Constant temperature bath adjustable to a temperature of 20°C ±1°C. The incubation bottles are kept in this bath during the 7-day duration of the test.

17.2.4 Storage of samples and removal of interferences

Sterile industrial effluents with a high oxygen demand and other properties (such as a high or low pH) that inhibit biochemical processes may be stored for 1 to 4 days after sampling, preferably at 4°C.

Non-sterile samples (containing effluents from paper mills, food processing factories and domestic sewage) should be tested as soon as possible after sampling. Delay due to transport and other causes should not exceed 1 day. Keep the samples at a temperature of 4°C.

The preparation of the sample is dependent on the presence and nature of interfering substances. These should be eliminated as instructed below. Shake the sample bottle before removing any part of its content.

Removal of active chlorine. Active chlorine is reduced in acid solution by the addition of an equivalent amount of sodium sulphite.

To 100 ml of the sample add 10 ml of the potassium iodide solution, 10 ml of the acetic acid and a few drops of the starch indicator. If a blue colour appears, titrate with the sodium sulphite solution (consumption a ml). The end point is indicated by disappearance of the blue colour.

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To a second 100-ml sample, add a ml of the sulphite solution and, after 10 min, the potassium iodide, acetic acid and starch solutions as above. If the solution turns blue add more sulphite (b ml) until the colour just disappears. Finally, add to a new portion of sample (a + b) ml of sulphite solution per 100 ml. This added volume should be noted for further consideration when the dilution factor is calculated. The sample is now ready for dilution.

Removal of sulphur dioxide. Under the conditions of the BOD determination sulphur dioxide consumes oxygen and must therefore be removed.

If the sample contains sulphur dioxide transfer a measured volume to a beaker and acidify with the sulphuric acid to pH3 or just below. Note the volume of acid used and take this into account when calculating the dilution factor. Pass nitrogen through the sample for about 30 min, by means of a glass tube at the lower end of which is connected a piece of fritted glass, or another suitable device for producing small bubbles of gas.

Check the immediate oxygen demand by titrating a known volume of the sample with standard iodine solution; 1 ml of 0.01 N iodine solution corresponds to 0.08 mg of oxygen. The immediate oxygen demand of the final test sample should not exceed 0.1 mg per litre.

Removal of hydrogen sulphide and other sulphides. Remove with nitrogen at pH5 or lower, as described above.

Removal of suspended matter. If the sample contains suspended matter it should be decided whether its biochemical oxygen demand is to be included in the test. If so, homogenize the sample in a mixer; if not, remove the suspended matter by sedimentation: shake the sample and transfer a part of it to a beaker or flask. After 30 min take the amount required from the supernatant solution.

The presence of suspended material in the test samples may lower the precision of the test. For example, if the object of the investigation is to study the performance of a biological treatment with regard to degradable, dissolved as well as suspended matter, suspended material should not be separated.

Removal of excess oxygen. If the sample contains oxygen in excess of the equilibrium amount at room temperature (about 9 mg/l) it should be removed by passing air through it.

If no interfering substances are present, or if these have been eliminated as instructed, dilute the sample as described below.

17.2.5 Dilution of sample

Although this method covers effluents containing a wide range of concentrations of oxygen-consuming material, the oxygen content at the end of the incubation period X_7 should be within 35 to 60 percent of the original value X_0, which is 8 to 9 mg per litre. The original sample will therefore normally have to be diluted, and several "test samples" of different dilutions must then be prepared from the original sample. The dilution is expressed in terms of a dilution factor, d, which is the ratio of the volume of the diluted sample to that of the original sample.

Prepare the test samples as follows:

Bring the samples and dilution water to a temperature of $20^{\circ}C \pm 1^{\circ}C$.

If the approximate oxygen demand of the original sample is unknown, prepare 3 or 4 test samples having dilution factors forming a series, the consecutive terms of which do not differ by more than a factor of 2.5. A suitable series is 1, 2.5, 5, 10, 25...

Transfer (with a pipette) to a 1-l volumetric flask a volume not less than 5 ml original sample, selected to give an oxygen content at the end of the incubation period, X_7, of between 3 and 5 mg per litre. If the dilution factor to be used is greater than 200, the dilution must be made in two steps. If the sample contains large amounts of suspended material, use measuring cylinders.

Prepare each test sample in a 1-l volumetric flask. Before diluting to the mark, add to the sample 1 ml of each of the nutrient solutions (para. 17.2.2) and 1 ml of the seed. Check that the pH of the solution is between 6 and 8, and adjust if necessary with a few drops of the 0.1 M sodium hydroxide solution or the 0.05 M sulphuric acid.

All dilutions shall of course be performed with the tested dilution water. The dilution water is introduced into the measuring flasks by means of a siphon. The outlet of the siphon hose should, all the time, be kept well below the water surface in the flask. This precaution is in order to prevent aeration of the sample; such an aeration could cause erratic results.

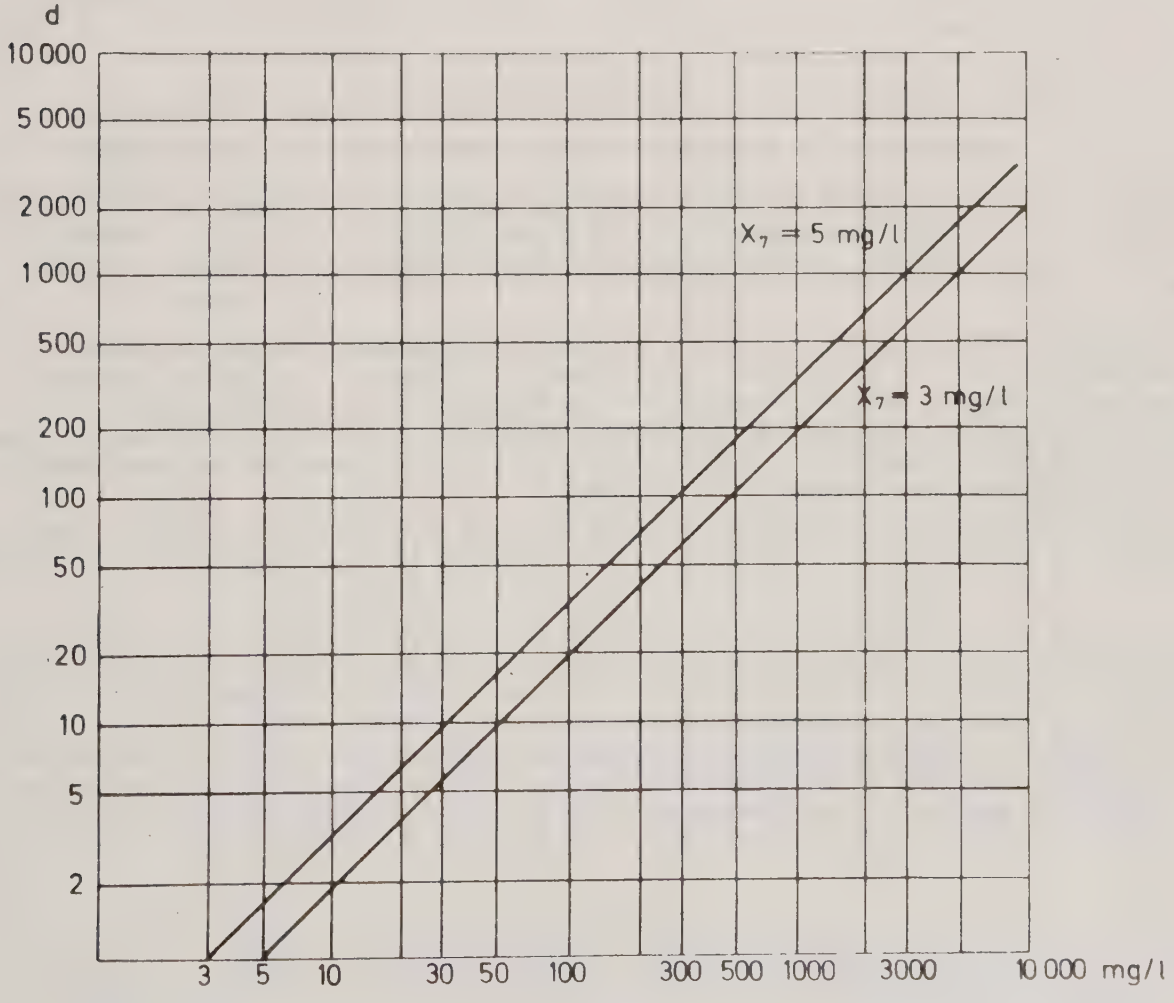

Figure 1. The dilution factor d plotted against the biochemical oxygen demand, BOD_7. The two lines correspond to values of X_7 of 5 and 3 mg/l, respectively

BOD dil 6

In selection of the dilution factors to be applied, the diagram in Fig. 1 may be used for guidance. The diagram illustrates the relationship between the BOD_7 for the original sample and the dilution factors to be selected for X_7=5 mg/l and X_7=3 mg/litre.

If the number of samples to be analysed is large it may be convenient to use dilution water containing 1 ml/l of each of the nutrient solutions.

If the pH is adjusted before the final dilutions, the properties of the sample may be altered; where possible, pH adjustments during the preparation of the sample should therefore be avoided.

17.2.6 Procedure

To check the procedure run 3 control samples parallel with each batch of test samples:

(i) a control sample containing 25 ml of seed per litre of dilution water. The sample has to be diluted.

(ii) a control sample containing 1 ml of seed and 15 ml of the glucose-glutamine solution per litre. The sample has to be diluted.

(iii) a reagent blank containing 1 ml of seed per litre.

All 3 control samples should contain the normal quantities of nutrient salts.

The BOD_7 of the seed (i) should fall within the range 50 to 300 mg O_2 per litre. Control sample (ii) should have a BOD_7 of 255 mg $\pm$10 mg of oxygen per litre. A reduction in the oxygen content of the reagent blank exceeding 0.7 mg of oxygen per litre indicates that at least one of the reagents is contaminated.

Incubation. The sample solutions are transferred to incubation bottles by means of a siphon as described in the preparation of dilutions. For each test or control sample, 4 incubation bottles are filled. Every bottle is rinsed by allowing approximately one bottle volume of the sample to overflow. The outlet of the siphon should be kept below the water surface all the time. The bottles should be filled to the brim and the stoppers inserted. Check that no air has been trapped in the bottles.

Place 2 of the bottles in a constant temperature bath at 20°C $\pm$1°C for 7 days. Exclude light to prevent growth of oxygen-producing algae.

Determination of oxygen. Determine the amount of oxygen in each of the 2 remaining bottles. Remove the stopper and deposit, on the bottom of the bottle, 1 ml of the alkaline iodide solution, and just below the surface 1 ml of the manganese sulphate solution. Replace the stopper, thereby expelling approximately 2 ml of the solution (this is corrected for in the calculation). Shake the bottle, leave for 5 min and shake again. Allow the precipitate to settle - this will take about 15-30 minutes. Remove the stopper carefully and add 3 ml of the phosphoric acid at the surface; replace the stopper immediately; about 3 ml of solution will be expelled (no correction is required). Shake the bottle until the precipitate is dissolved.

Transfer the contents of the bottle to a titration flask. Titrate immediately with the 0.01 N sodium thiosulphate solution to a pale straw colour. Towards the end of the titration add a few drops of the starch solution as an indicator and continue carefully, until the sample is colourless. Calculate the oxygen content from the expression:

$$X_0 = \frac{8\,000 \cdot a \cdot n}{b - 2}$$

where

a = volume of sodium thiosulphate solution consumed by the sample, millilitres

b = volume of the incubation bottle, millilitres

n = normality of the sodium thiosulphate solution, equivalents per litre

X_0 = oxygen content, mg of O_2 per litre

Record the mean of the two samples with 3 significant figures, as the oxygen content.

Determine the oxygen content in the incubated bottles after 7 days $\pm$6 h and record the mean as X_7.

17.2.7 Calculation

Calculate the oxygen demand from the expression

$$BOD_7 = d\left[(X_0 - X_7) - (C_0 - C_7)\right]$$

where

X_0 = oxygen content in the test sample at the start, mg of O_2 per litre

X_7 = oxygen content in the test sample after 7 days, mg of O_2 per litre

C_0 = oxygen content in the reagent blank at the start, mg of O_2 per litre

C_7 = oxygen content in the reagent blank after 7 days, mg of O_2 per litre

d = dilution factor, the ratio of the volume of the diluted sample to that of the original sample

BOD_7 = biochemical oxygen demand, mg per litre

For calculation of the dilution factor, the entire pre-treatment of the sample must be taken into consideration. If, for instance, 14 ml sodium sulphite solution for removal of active chlorine is added to 100 ml of the original sample, this results in a dilution of 114:100=1.14 times. Then 20 ml of this treated sample is diluted to 1 000 ml with the tested dilution water, resulting in a dilution of 1 000:20=50 times. The overall dilution factor is then 1.14x50=57.

17.3 Biochemical oxygen demand of waste water - Manometric method

17.3.1 Introduction

This method is designed to determine BOD in undiluted (or slightly diluted) waste waters and effluents, where the course of oxygen consumption is registered as a time function. The instrumentation is too costly for this method to be suitable for routine analysis of a great number of samples, but for special studies of waste waters or effluents of interest, the manometric procedure offers some advantages. For a further discussion of this see Chapter 6.

The biochemical oxygen demand of a water sample is the amount of oxygen that is consumed in biochemical oxidation of organic matter under specified conditions.

The purpose of this determination is to measure (in an indirect way) the amount of organic material decomposed and oxidized by natural aquatic micro-organisms. Under the conditions of test, however, several inorganic compounds or ions may consume oxygen; these substances are removed before the test is started.

BOD man 2

The principle of the method is as follows: The pre-treated sample is seeded with domestic sewage containing nutrient salts to initiate biochemical oxidation.

The sample is placed in an apparatus intended for the manometric determination of BOD. The oxygen is available partly as dissolved oxygen in the sample, partly in air above the sample. When the dissolved oxygen is consumed through biochemical oxidation it is replaced from the gaseous phase above the sample, creating a pressure drop as the carbon dioxide formed during the biochemical oxidation is absorbed in an alkaline media.

Apparatus available work on one of two principles. According to the first one, the consumed oxygen is replaced by generation in an electrolytic source, while the generated amount is registered. This apparatus functions at constant oxygen pressure, giving a first order reaction mechanism. A recently developed apparatus for biochemical oxygen demand is the Sapromat, which is designed as a six- or twelve-channel device working according to the respirometric method. Oxygen consumed during respiration in a closed vessel is generated electrolytically under standard conditions, and the amount of oxygen generated over a given period is recorded on a printer. With this device, it is possible to follow the oxygen consumption from time to time. From the data obtained via the recorder, a biochemical oxygen consumption curve may be constructed, which will give the ultimate value of biochemical oxygen demand. The shape of the curve reflects disturbances like nitrification, inhibition due to toxicity or mechanical leakage. The rate of oxidation can easily be estimated.

In the other type of apparatus the consumed oxygen is not replaced. The oxygen consumption is measured as pressure drop by a manometer. The kinetics of the measurement may be described as a second order reaction.

17.3.2 Reagents

Check the quality of all the distilled water used in this method.

Distilled water sometimes consumes oxygen. The quality of the water may be checked by the determination of the permanganate number of the water directly and after it has been boiled for 20 minutes. If the difference in the consumption of sodium thiosulphate solution (0.02 N) exceeds 0.08 ml, the water should not be used for preparing solutions.

Nutrient solutions:

(a) Phosphate buffer. Dissolve separately in sterile, distilled water, 34 g potassium dihydrogen phosphate, KH_2PO_4, 87 g dipotassium hydrogen phosphate, K_2HPO_4 and 133.6 g disodium hydrogen phosphate, $Na_2HPO_4 \cdot 7H_2O$. Mix the solutions and dilute to 1 l with sterile, distilled water.

This nutrient solution is susceptible to bacterial contamination and has a short shelf life. The water used for its preparation must be sterilized by boiling it a few minutes; also sterilize the storage bottle by heating it for 2 h at 120°C. The solution should be renewed once a month or if it becomes turbid.

Do not dip unsterilized objects such as pipettes into the solution. Do not return unused portions to the storage bottle.

(b) Nitrogen nutrient solution. Dissolve 216 g potassium nitrate in distilled water and dilute to 1 litre.

(c) Magnesium sulphate solution. Dissolve 22.5 g magnesium sulphate, $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 litre.

(d) Iron (III) chloride and calcium chloride solution. Dissolve separately 0.25 g of iron (III) chloride, $FeCl_3 \cdot 6H_2O$, and 27.5 g of calcium chloride, $CaCl_2$, in distilled water. Mix the solution and dilute to 1 litre.

These nutrient solutions are more concentrated than the corresponding solutions in the dilution method for BOD. The use of concentrated solutions here is essential, because the two apparatus are capable of determining much higher BOD values in the tested sample portion than is possible in the dilution method.

Seed. Domestic sewage, preferably taken from the effluent, from the primary clarifier in a sewage treatment plant. Before use, leave the seed in contact with air in an open vessel for 24 to 36 h at 20°C. Take a portion of the decanted seed with a pipette and filter through surgical cotton or paper filter.

To avoid infection of personnel, extreme care should be exercised when handling the seed. Rinse pipettes and other glassware thoroughly after each test. Do not place used pipettes on laboratory benches, but use a pipette stand. After working with seed disinfect the hands. At the end of each working day sterilize all the glassware.

Seed solution. Take 10 ml of nutrient solutions (a) and (b) and 5 ml of solutions (c) and (d), to each litre of filtered seed.

Dilution water. Distilled water, free from copper and having an oxygen content, at 20°C, of 8 to 10 mg/l, obtained by bubbling air through the water.

Sulphuric acid, 0.05 M. Add carefully 2.8 ml of concentrated H_2SO_4 to 700 ml of distilled water and dilute to 1 litre.

Sodium hydroxide solution, 0.1 M. Dissolve 4 g sodium hydroxide, NaOH in water and dilute to 1 litre.

Glucose-glutamine solution. Dissolve separately in distilled water 150 mg glucose, $C_6H_{12}O_6$, and 150 mg glutamic acid, C_5HgNO_4 (both substances dried over phosphorus pentoxide). Mix the solutions in a 1-l volumetric flask and dilute to the mark. The solution should be freshly prepared.

17.3.3 Equipment

- Apparatus for the manometric determination of BOD, e.g., "Sapromat", manufactured by Voith GmbH, Heidenheim, Fed.Rep. of Germany (capable of handling an oxygen consumption rate of 45-90 mg oxygen per litre and hour) or the simple apparatus developed by Hach Chemical Co., Ames, Iowa, U.S.A. (The total BOD measurable with this equipment is about 300 mg/litre.)

- Constant temperature chamber adjustable to a temperature of 20°C ±2°C.

17.3.4 Storage of samples and removal of interferences

See dilution method (para. 17.2.4).

17.3.5 Procedure

Dilution of the sample. The degree of dilution is governed by the working range capacity of the apparatus. Thus the sample should be diluted according to the manufacturer's instructions.

Determination of BOD. The relation of volume of pre-treated sample to volume of seed solution should be between 8-10:1. A decision on the amount of seed necessary in the determination requires certain experience or even pre-investigation.

Add a suitable amount of seed solution to the pre-treated and diluted sample. Measure the suitable volume and transfer to the measuring unit. Run separately a sample of seed solution. Place the absorptive material for CO_2 into the special container in the measuring unit. Start the measurement according to the manufacturer's instructions. If the apparatus is not equipped with a continuous registering unit, read off the measuring unit (manometer) twice during the first two days of the determination. Check the air pressure at the same time.

Leakages can be revealed at low BOD values by plotting the BOD value and the air pressure. There will be a leakage in the manometer if low BOD values are observed at falling air pressure and high BOD values at increasing air pressure. The conditions will be the opposite if leakage occurs in the measuring unit.

Check the apparatus occasionally by determining the 7-day BOD of the glucose-glutamine solution with 10 percent seeding. This should be 220 $\pm$10 mg per litre.

17.3.6 Calculation

Calculate the biochemical oxygen demand of the sample from the expression

$$X = \frac{a - (b \cdot d)}{c}$$

where

X = BOD of the sample (diluted), mg/l

a = registered value, mg/l

b = part of seed in the sample

c = part of sample

d = BOD of the seed solution, mg/l

Example:

20 ml of seed solution (part = 0.1)
180 ml of sample (part = 0.9)
registered value = 195 mg/l
BOD of seed solution = 150 mg/l

$$X = \frac{195 - (0.1 \cdot 150)}{0.9} = 200 \text{ mg/l}$$

Report the results to two significant figures.

17.4 Determination of the suspended matter content of water

17.4.1 Introduction

For the purpose of this method suspended matter in natural water and waste water is defined as the matter, per unit volume, retained when a sample of the water is filtered through a specified filter. (Floating material and supernatant layers of nonaqueous liquids should be removed before the filtration.) The sample is filtered through a glassfibre filter and the retained dried matter is determined by weighing.

While the test is applicable to most kinds of effluents, special care is required when filtering waters containing suspended matter that build up a filtering layer having a smaller pore size than the actual filter. The method is not intended for the determination of suspended oils.

Many effluents, for example from pulp, paper or wallboard mills, contain minute particles which can block the filter by adhering to the walls of the pores, thus diminishing the pore size. When this happens the filtration time will be prolonged and the results will vary with the sample volume taken for analysis. In this case filter two samples, one at least twice the size of the other, but keep both rather small. Report the two results and the sample volumes and state that the results are approximate.

17.4.2 Equipment

- Glassfibre filter, pore size approximately 1 µm, 55 mm in diameter. A suitable filter is "Whatman GF/C", pore diameter about 1 µm. When the sample contains suspended fibres, filters with pore diameter around 10 µm may be used. Filters made of cellulose fibres, such as Schleicher and Schüll 589 or Munktell OOR, are commonly used in routine control. If such filters have been used, this should be stated. The results will then not be reported as being obtained in accordance with this standard method.

- Büchner funnels, with an inner diameter to take the filters.

- Filter pump and filter flask, for the Büchner funnels.

- Drying oven, adjustable to 105°C ±5°C.

- Analytical balance, accuracy ±0.1 mg.

17.4.3 Sampling and sample storage

Remove floating material and other particulate agglomerates not dispersed on agitating the gross sample. Allow the sample to reach ambient temperature.

Because some kinds of effluent are unstable, the samples should be analysed without delay.

17.4.4 Procedure

Wet a filter in the Büchner funnel with distilled water and apply suction for a few seconds. Remove it carefully and dry it for 1 h at 105°C ±5°C in the drying oven. Allow it to attain equilibrium with the atmosphere near the balance and weigh to the nearest 0.1 milligrammes. Ensure that during this procedure the filter is not contaminated by dust.

Place the weighed filter in the Büchner funnel and apply suction. Moisten the filter with a few drops of distilled water and check that it fits the funnel tightly.

Agitate the gross sample vigorously and transfer immediately (and in one portion) a suitable volume (generally about 100 ml and in no case less than 25 ml) to a measuring cylinder. The volume taken should contain 5 to 100 mg of suspended matter and should be chosen so that the filtering time is less than 1 minute. Read off the sample volume on the cylinder scale.

Transfer the sample quantitatively to the funnel. If the time required for the sample to pass the filter exceeds 1 min, record the time needed. Rinse the cylinder with 20 ml of distilled water and use this for washing the filter. Rinse the sides of the funnel with a few millilitres of distilled water. If the sample is salt or brackish water, wash the filter thoroughly with about 100 ml of distilled water.

Continue suction until the filter is almost dry. Remove it carefully from the funnel with tweezers and dry it in the drying oven for 2 h at 105°C ±5°C. Allow to cool, and weigh as before. Check that the temperature and relative humidity near the balance have not changed appreciably from the time the filter was weighed prior to filtration.

Carry out the procedure in duplicate. The results should agree to within 10 percent of the mean value.

17.4.5 Calculation

The suspended matter content is given by the expression

$$X = 1\,000\,\frac{(a-b)}{c}$$

where

a = weight of filter and residue, mg

b = weight of filter, mg

c = volume taken for analysis, ml

X = suspended matter content, mg/l

If X is at least 5 mg/l, report to two significant figures; otherwise report the result as "less than 5 mg/litre".

17.5 Determination of small amounts of "non-polar" hydrocarbons (mineral oil) in natural waters

17.5.1 Introduction

This method is designed for the determination of low concentrations of mineral oil in natural water, especially sea water. However, with some modifications (given in the text) sewage water and industrial effluents may be also analysed regardless of whether the concentration of oil is low or high.

The mineral oil, from an acidified sample, is extracted with carbon tetrachloride. After separation from disturbing substances by column chromatography on aluminium oxide, the concentration of the oil in the extract is determined by measurement of its absorption of infra-red radiation. The sample is acidified to prevent saponification of any fatty material present, thus minimizing the extraction of fat. The presence of acid also promotes the extraction of the mineral oil.

Mineral oils consist of mixtures of hundreds of hydrocarbons, mainly alkanes (paraffins), alkenes (olefins) and aromatics. Thus in routine analysis it is impossible to perform quantitative determinations of even the dominating, individual hydrocarbons. Being rather insensitive to the aromatic compounds, the infra-red photometric technique determines the paraffinic and olefinic constituents of the mineral oil. As the infra-red measurement is performed at the absorption frequences for the CH-, CH_2- and CH_3- groups in the molecules, this method is not specific for mineral oil. To minimize the disturbances, fat, humic substances and other compounds are removed by column chromatography on aluminium oxide. Thus the concept of "mineral oil" is in this method redefined in the terms of the working procedure:

> Mineral oil hydrocarbons - all substances containing CH-, CH_2- or CH_3- groups (no matter whether they are pure hydrocarbons or derivatives of any kind) which are not retained by aluminium oxide and which show infra-red absorption bands at 3.38 µm (2 960 cm^{-1}) and 3.42 µm (2 925 cm^{-1}).

In the chromatography step, polar substances are separated from the non-polar mineral oil constituents. However, the terms "polar" and "non-polar" must here be put in quotation marks as they do not entirely conform with the classical concepts. A non-polar substance (in the classical meaning), such as benzene, passes the chromatographic column used here, while phenantrene (which is also non-polar in the classical meaning) is quantitatively retained. Thus a certain part of the mineral oil may be lost on the chromatographic column, the proportion depending on the type of oil. With the use of infra-red spectroscopy only, it is not possible to claim that the "non-polar" hydrocarbons found in the sample originate

from mineral oil. Thus, if further information on the samples is not available, the results of the analysis should be reported as "total amount of extractable "non-polar" hydrocarbons of mineral oil type".

17.5.2 Sampling equipment and techniques

Because of the risk of contaminating the sample, it is essential that the equipment used should be of a construction that never requires lubrication; perspex, PVC, glass, etc. are suitable materials.

After sampling, the water is transferred to clean glass bottles (preferably with ground stoppers; avoid touching the grounded surfaces).

All equipment used should be carefully washed and thoroughly rinsed with large amounts of ion-exchanged or distilled water. Whenever possible the different items should be finally rinsed with carbon tetrachloride.

A stainless steel bucket (10 l) can be used to collect the surface samples (about 2 l). This sampling of the sea surface is always performed from the moving ship, just before it stops at a sampling station. This method seems sufficient to prevent contamination from the ship itself. The samples from the deeper water layers can be obtained with a TPN (Total Plastic Nansen) water sampler (capacity 1.7 l) obtainable from Hydro-Bios Apparatebau GmbH, Kiel, Fed.Rep. of Germany. The TPN should never be fastened directly to a hydrographic wire but to a 5-m nylon rope attached to the end of the wire. The TPN is messenger-operated; this is done with the help of a common messenger-operated water sampler placed so that it overlaps the joint between the wire and the rope.

For sampling from shallow depths at sea or in lakes and rivers, a Ruttner type water sampler is handy to use.

17.5.3 Storage and preservation of samples

The sample water is transferred to the cleaned glass bottle (2 l). (Plastic bottles should not be used for storage, as the oil will be adsorbed by the walls of the bottle, to a greater or lesser extent.)

For preservation 1.5 ml (per sample) of a saturated solution of mercuric chloride ($HgCl_2$) can be added. Samples preserved in this way and stored in darkness at room temperature have been shown to keep an almost constant oil concentration for four weeks. Instead of the mercuric chloride, sodium azide (NaN_3) can be used. In that case 1 g/l sample is added. Due to lack of experience in this method no maximum tolerable storage time can be stated.

If impure carbon tetrachloride is used to rinse the sample bottle, it must be dried before the sample is introduced. The impurities in any remaining carbon tetrachloride will disturb the analysis.

17.5.4 Reagents

Hydrochloric acid, analytical grade. The concentrated acid (sp.gr. 1.19) is diluted with an equal volume of distilled water.

Carbon tetrachloride. It is not important to use expensive spectroscopic or certified grades. It is sufficient if the quality used is free of any significant absorption maximum in the infra-red region between 3 333 cm^{-1} and 2 500 cm^{-1} (3-4 μm). The quality of each bottle of solvent should be checked (see Fig. 2). The same quality - preferably from the same bottle - is used through all steps of the analysis. A technical quality of the solvent can in many cases be used after cleaning, by shaking for 1 h with activated carbon (100 ml carbon granules/litre of solvent) and subsequent filtration.

Oil 3

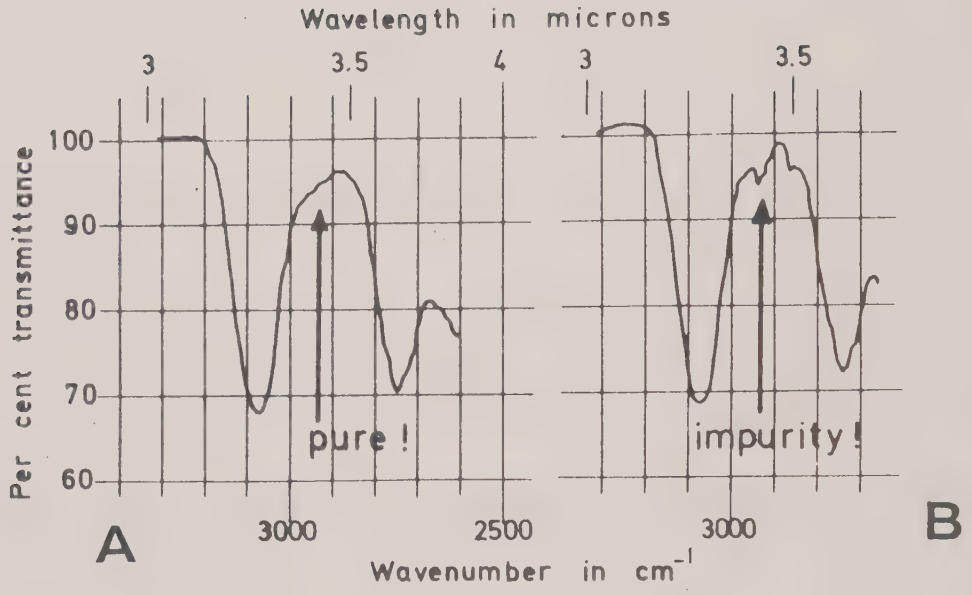

Figure 2. Control of the purity of the carbon tetrachloride. Spectra were obtained at double beam mode of the instrument with an empty cell as reference.
(a) clean solvent with no peak at about 2 960 cm^{-1}
(b) presence of a slight, but tolerable impurity

Aluminium oxide, neutral, activity step 1 according to Brockman. Type 507 C from Fluka (Switzerland) is quite suitable.

n-Hexadecane (cetane), analytical grade.

iso-Octane, analytical grade.

Benzene, analytical grade.

17.5.5 Equipment

- The samples are extracted in the glass bottles mentioned above. To collect the carbon tetrachloride after extraction, the stopper is replaced with a TS-cone provided with a stopcock (e.g., Quickfit Stopcock Adapter MF11, Fig. 3).

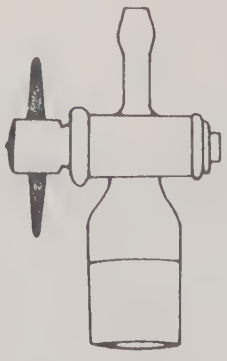

Figure 3. Stopcock adapter

- Thus the bottle acts as a separatory funnel. (When extracting with 25 ml it is not necessary to provide the adapter with an air inlet.) The presence of any kind of fatty lubricant, except the sample itself, will disturb the analysis, giving high, positive values. The use of stopcock keys made of PTFE is therefore recommended.

- Shaking machine with a reciprocating motion (e.g., from Edmund Bühler AG, Tübingen, Fed.Rep. of Germany, model No. Sm 2).

- Chromatography columns, 10 mm inner diameter. Either of the two types shown in Fig. 4 can be used.

- Glasswool.

- Measuring cylinders, capacity 25 ml.

- Filter paper. Munktell No. OOR, Whatman, No. 41 or 31 or equivalent.

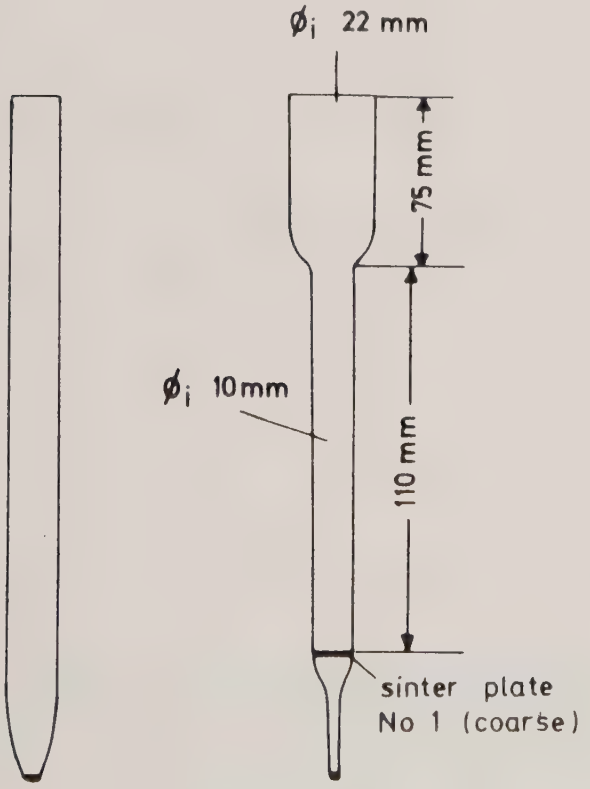

Figure 4. Chromatography columns

Figure 5. Adapter for Gooch Crucibles

- Funnels for filtering. To reduce the losses of carbon tetrachloride due to evaporation, a high, narrow type should be used. One suggestion is Pyrex No. 3960 (Adapters for Gooch Crucibles) or similar (Fig. 5).

- Centrifuge, capacity 50 ml in each tube, and capacity of 3 000 rpm.

Oil 5

- Infra-red spectrophotometer (IR-photometer). Any low- or medium-priced instrument will do if it has double-beam operation and capacity for 40 mm cells. Anyhow, an instrument with a grating monochromator is preferable.

- Cells, 40 mm Infrasil or equivalent quartz material.

17.5.6 Calibration

Obviously the best way to carry out the calibration is to prepare the calibration graph for the infra-red photometric determination based on the same oil as is present in the samples. In most cases, however, the identity of the oil is not known. In such cases a compromise must be accepted. Simard et al. (1951) proposed a standard mixture of 37.5 percent (by volume) of n-hexadecane, 37.5 percent of i-octane and 25 percent of benzene. (The adequacy of this mixture was further studied by Scholl and Fuchs (1968); see also para. 17.4.8.)

It has been shown by Lindgren (1957) and also by Carlberg and Skarstedt (1972) that generally one extraction is sufficient to recover more than 95 percent of the oil present in a water sample. Therefore it is not necessary to add the calibration oil, or standard mixture, to water samples and then extract and carry out the entire analysis as described below. It is sufficient to dissolve an accurately weighed amount of the oil (or standard mixture) in carbon tetrachloride (for instance about 100 mg/100 ml) and then dilute with the same solvent to prepare a series of solutions of known concentrations. If 25 ml of solvent is used for the extraction of the samples, it may be practical to prepare these calibration solutions in 25-ml measuring flasks. The absorbances of these solutions are then plotted against their concentrations expressed as mg/25 millilitres.

The correctness of this calibration procedure was confirmed by application of this method when analysing effluents from some factories. The concentrations of oil in these samples were so high that they had to be diluted about 100 times. Gravimetric determinations were made in parallel on several of the extracts after IR-photometric determination.

The following figures are representative for the results obtained:

Industry	Oil mg/l IR-photometric	Oil mg/l gravimetric
A	324	319
B	138	140
C	142	142

Thus the simplified procedure might be regarded as sufficient.

17.5.7 Procedure

Extraction. Add 2 ml of the diluted hydrochloric acid to the sample (approximately 2 l). Check that the pH is about 3 or less. Add 25 ml carbon tetrachloride with a measuring cylinder. Stopper the bottle and shake it by hand for a few seconds. Open the bottle and restopper it again. Repeat this procedure until the sample is entirely free of gas. Place the bottle (bottles) in the shaking machine for 60 minutes. A suitable speed is about 180 strokes (5-6 cm) per minute. The Bühler machine is capable of shaking four 2.5-l bottles at a time.

If considerably larger volumes are analysed, or if the oil concentration is high, it may be advisable to extract with 50 ml of solvent. For the analysis of water other than sea water, sodium chloride (50 g/l) must be added.

After the extraction stand the sample aside to let the phases separate. If an emulsion has formed or if the phases do not separate completely after two hours it is necessary to centrifuge.

Replace the stopper with the stopcock adapter described above. Secure it to the bottle with a clamp or rubber band. Invert the bottle and place it in a stand (for instance as in Fig. 6). Drain off the organic phase directly into the narrow filter funnel. The filter paper and the funnel should previously have been washed with 10-15 ml carbon tetrachloride. The filtered sample is collected in a 25-ml conical flask with a ground stopper.

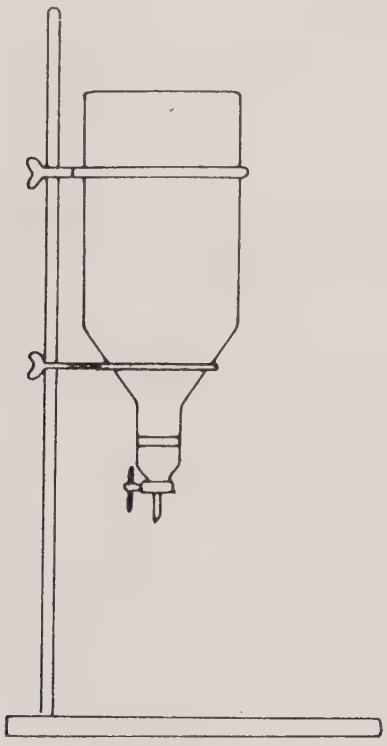

Figure 6. Glass bottle with stopcock adapter in the stand with rings

If it is necessary to centrifuge the emulsion and the organic phase, drain them directly into the centrifuge tube. After centrifugation, collect the organic phase from below the water layer with a pipette and filter. The extract is now ready for column chromatography.

If the oil content of the sample is very high, it may be necessary to extract twice or more.

When analysing not more than 2 l of sample in a 2.5-l bottle, it is generally possible to draw off the organic layer without having an air inlet to the bottle. However, if much emulsion is formed, it may be necessary to invert the bottle and let air in before the entire extract can be collected.

If centrifugation is not enough to separate the phases, the emulsion may be destroyed by vigorous shaking in a separatory funnel of the carbon tetrachloride and emulsion together with a water phase containing magnesium chloride in a suitable concentration (up to a saturated solution).

Oil 7

Column chromatography. This step is necessary in order to remove the "polar" substances from the sample.

Pour aluminium oxide powder into the column, to a height of about 10 cm. (If a column without a sinter plate is used, a small piece of glasswool must first be placed at the bottom of the column.) Do not tap the column in an attempt to pack the powder, because this will inevitably result in an extremely slow flow rate through the column. Place a small piece of glasswool on top of the oxide. This will prevent the powder from whirling up when pouring in the sample.

Now fill the column with carbon tetrachloride and let about 6 ml flow out into a measuring cylinder. This is approximately equal to the volume retained by the oxide. When the column has stopped dripping, carefully pour a part of the sample onto the column. When another 6 ml has passed out (use measuring cylinder), collect the remaining part of the sample as it passes the column. Twentyfive millilitre grounded conical flasks are very suitable for this purpose.

Infra-red spectrophotometric determination. The reference and sample cells are carefully cleaned (inside and outside) with carbon tetrachloride and then filled with the same solvent. The absorbance is checked in the region 3 333 cm^{-1} to 2 500 cm^{-1} (3-4 μm). The recorded spectrum should just be a line, which is horizontal and free from peaks.

The sample cell is filled with the sample and the spectrum recorded in the same spectral region. If the absorbance of the sample appears to be greater than is suitable, according to the calibration curve, the sample has to be diluted with carbon tetrachloride and the spectrum recorded once again.

It is recommended that the photometric measurement be carried out twice for every sample: before and after the column chromatography. The first measurement represents the total amount of carbon tetrachloride extractable organic substance. The second represents the total amount of carbon tetrachloride extractable "non-polar" hydrocarbons of mineral oil type. As this second quantity should be smaller than (or equal to) the first one, this is a good way to check that the extract has not been polluted during the major part of the laboratory work. Secondly, if the extract is lost or destroyed for some reason, the first measurement gives a maximum value for the oil content.

17.5.8 Calculation, precision and accuracy

To evaluate the spectra obtained, a straight line is drawn between the two absorption minima (that means about 100 percent transmission) surrounding the region 3 000 -2 800 cm^{-1} (see Fig. 7). This line is normally horizontal. It is not unusual, however, for a slight absorption to remain in the region of 2 500 cm^{-1} even if the recorded curve there is a straight line. This is due to increasing absorption in the carbon tetrachloride and the cells. In such a case, a horizontal line is drawn from the minimum in the 3 300-3 000 cm^{-1} region. The spectrum can now be evaluated in two ways. If calibration is done using the same oil as is present in the sample, it is sufficient to measure the absorption maximum at 2 925 cm^{-1} from this baseline.

If, however, the standard mixture (or another oil) is used for the calibration, it may be more correct to measure the absorption maxima at 2 960 cm^{-1}, 2 925 cm^{-1} and 2 850 cm^{-1}, calculate the corresponding absorbances and use the mean value of the three. In this case the calibration curve must of course be prepared using the same evaluation technique. Most instruments are graduated in percent transmittance and in that case the transmittance value obtained is converted into absorbance by means of a standard table or the formula:

$$A = \log \frac{I_o}{I}$$

where

A = absorption

I_o = intensity of incident radiation (100 percent)

I = intensity of the radiation passing through the sample (in percent)

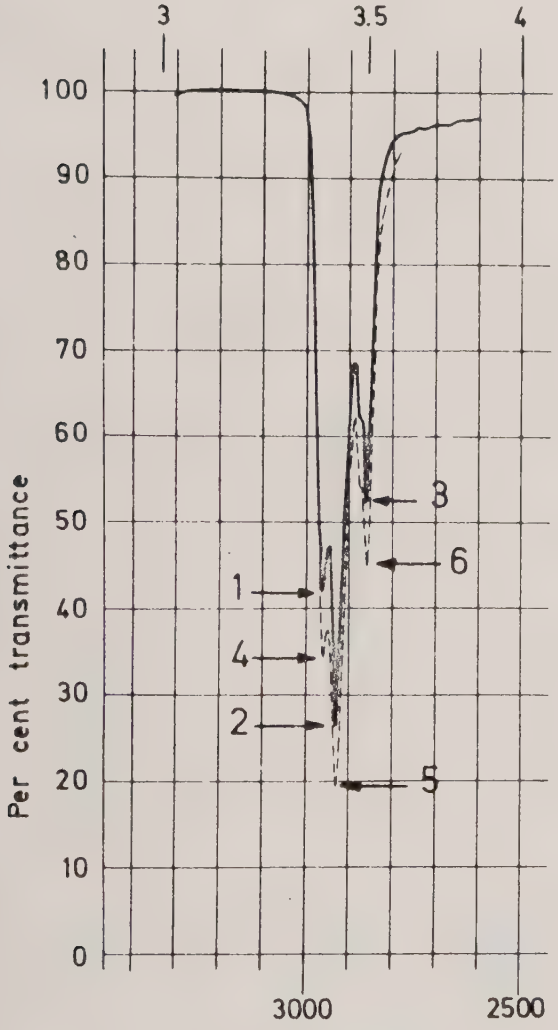

Figure 7. Interpretation of spectrum. Two methods are possible:

for the upper curve (chromatographed sample of fuel oil):

Peak No.	Percent transmittance	Absorbance
1	41.0	0.387
2	26.0	0.585
3	52.0	0.284
	Mean value	0.419

or just use the value of peak No. 2

in the same way for the lower curve (the same sample before chromatography):

Peak No.	Percent transmittance	Absorbance
4	34.0	0.469
5	19.0	0.721
6	44.8	0.349
	Mean value	0.513

or just use the value of peak No. 5

See also the text for further explanation.

To obtain a general formula for calculating the final concentration of the oil, it is suitable to construct a calibration curve with absorption plotted versus mg oil/25 ml carbon tetrachloride (Fig. 8) and to regard this as the result of an extracted 1-l sample.

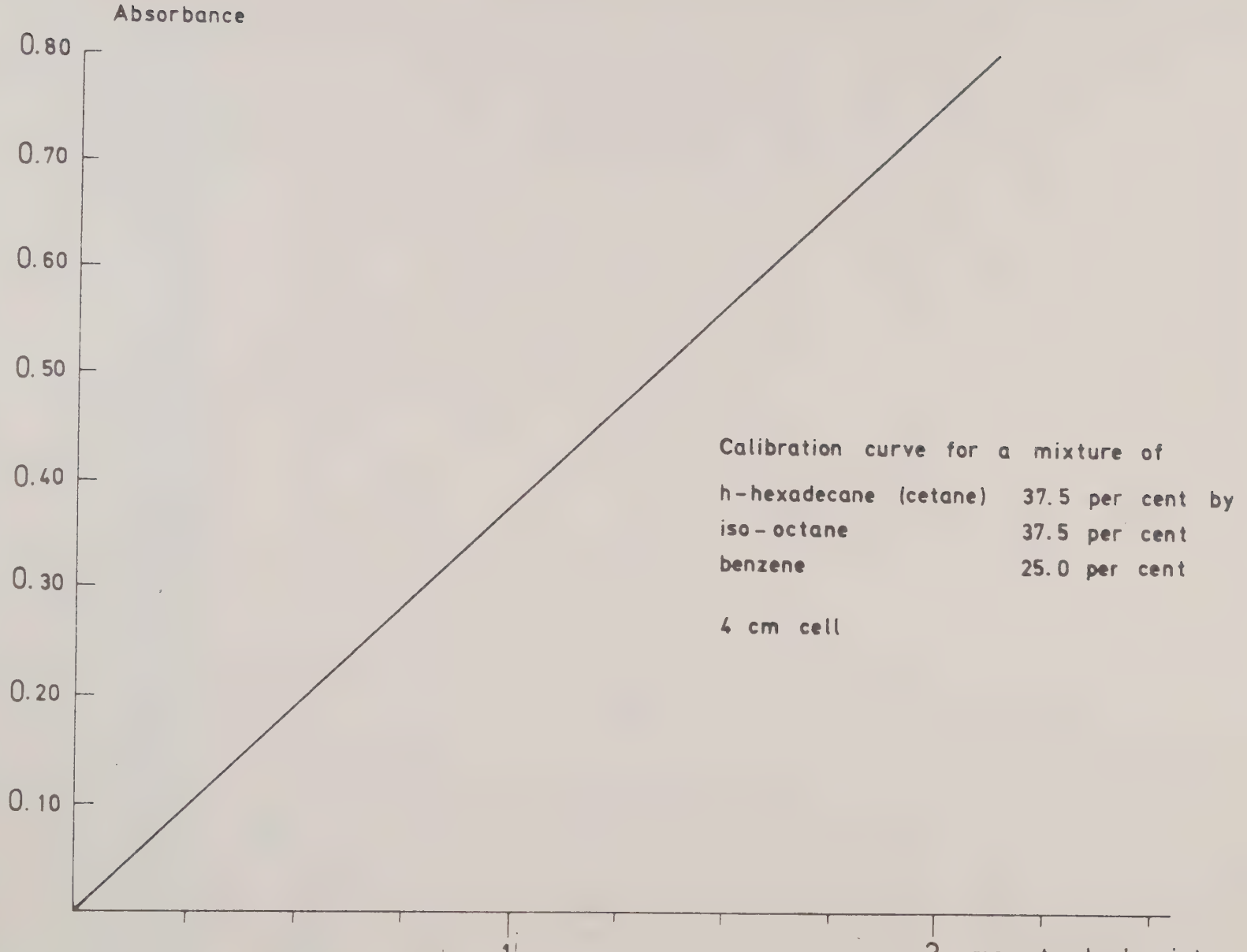

Figure 8. Calibration curve for a mixture of
h-hexadecane (cetane) 37.5 percent by volume
iso-octane 37.5 percent
benzene 25.0 percent
(4-cm cell)

In that case the formula will be:

$$C = \frac{a \cdot ml\ CCl_4}{25} \cdot \frac{1\,000}{b} \cdot d$$

where

C = mg/l hydrocarbons, oil

a = mg/25 ml from the calibration curve

ml CCl_4 = total volume of carbon tetrachloride used for the extraction of the water sample (this includes possible multiple extractions)

b = ml sample used in the analysis

d = possible dilution factor in the photometric step

The result is reported as "non-polar" hydrocarbons, mg/l, or as oil mg/l if sufficient information is available on the sample.

At present 0.05 mg of the standard mixture (or oil) per litre of sample is regarded as this detection limit for the procedure described above.

For precision and accuracy, one must regard the analysis as consisting of two parts; laboratory procedure with extraction, chromatography, etc. and the calibration.

As mentioned above, it has been found that one extraction is generally sufficient to recover more than 95 percent of the oil in the sample. It has also been found that when analysing multiple samples at the concentration level 0.30 mg/l, the values agreed to within $\pm$7 percent of the mean (that means a deviation of $\pm$0.02 mg/l). Infra-red measurements of five samples of fuel oil in carbon tetrachloride gave, after column chromatography, a deviation of 2.5 percent from the mean value. Thus it can be concluded that the precision and accuracy of the analytical procedure is good.

As pointed out earlier, the most critical link in the chain is the calibration: which standard to choose and how to evaluate the spectra. Because different types of oil have different specific absorptivities ranging from 1.75 to 2.99 $l \cdot g^{-1} \cdot cm^{-1}$ for lubricating oil, drilling oil and fuel oil respectively (Scholl and Fuchs, 1968) they would give calibration curves with different gradients. Obviously the standard mixture method is an acceptable compromise between the extremes mentioned. The specific absorptivity of this mixture is 2.37 $l \cdot g^{-1} \cdot cm^{-1}$ and thus the resulting accuracy is $\pm$26 percent if the most extreme oils are included.

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Paragraph 17.1 has been compiled by S. Carlberg on the basis of a method published by The Scandinavian Pulp, Paper and Board Testing Committee (SCAN-W 1:66). Paragraph 17.2 is based on paper SCAN-W 5:71 and has been contributed by P. Solyom, who also compiled paragraphs 17.3 and 17.4, the last one being based on the relevant standard Swedish method published by Sveriges Standardiseringskommission (SIS 02 81 12, Environmental test methods; Determination of suspended matter content of water). This method is equivalent to the Danish standard method DS 276 and the Norwegian standard method NS 4733. Paragraph 17.5 has been contributed by S. Carlberg based on Carlberg and Skarstedt (1972); Simard et al. (1951); Lindgren (1957); and Scholl and Fuchs (1968).

18. METHODS FOR SAMPLING AND TREATMENT OF BIOLOGICAL MATERIAL

18.1 Field sampling of biological material for determination of toxic substances

18.1.1 Introduction

Samples should be kept deep frozen between the catch and the laboratory treatment. The material should thus be sampled directly for the investigation, preferably by research vessels or at least by research personnel on board commercial vessels. However, since the organisms often have to be bought at the fish market, they may have been dead for some time and the processes of drying and decay may have begun, followed by alterations in the concentrations of the substances to be investigated. If this is the case, information must be obtained as to the treatment of the material and length of time since the catch.

Not very much is known about the changes that occur in the concentrations of the toxic substances when comparing tissue frozen some time after the catch to that deep frozen directly. However, it is known that rapid post mortem degradation of DDT takes place in liver tissues (Jefferies and Walker, 1966). On the other hand, 5 months of outdoor exposure of seal blubber have shown no significant changes in the DDT and PCB levels (Olsson et al., 1974). These changes depend partly on the climate, the manner of storage, the time elapsed after catch, etc. A day's storage without deep freezing can hardly be expected to alter appreciably the concentrations of most pollutants, but if the tissue has lost much water, for example, this can lead to considerable changes in the concentration per unit weight.

18.1.2 Algae and invertebrates

Algae collected for the determination of toxic substances must be washed, preferably in water from the same environment, to get rid of particles, etc. Then the algae should be deep frozen, and when analysed - as soon as possible - the water content should be determined so that the concentration of the pollutants can be expressed on dry weight basis.

Since it is impossible to remove all water adhering to the bodies of fresh algae, it is consequently impossible to get exact concentration values on wet weight basis. Dried algae from herbaria can be examined but the results of the analyses must be very carefully interpreted since certain toxic substances may have entered the tissue during storage.

Smaller invertebrates (e.g., hydroids, polychaetes, bryozoans) should also be washed but can afterwards be deep frozen and stored for a long period. The determination of concentrations are generally most safely done on dry weight basis.

Plankton sampling and analysis are especially difficult. In most cases the concentrations of toxic substances must be determined (on dry weight basis) for a mixture of species which varies from place to place, and according to depth and time. Determinations on wet weight basis are less reliable due to the water adhering to the tiny plankton organisms, but might be valuable for relative comparisons between samples.

Invertebrates and plankton should be stored in glass or plastic jars (glass, for examination of organochlorines); when plastic is used, this should be of the propylene type (not styrene).

It is much easier to use larger animals for studies of the concentrations of toxic substances, thanks to the possibility of cutting out pieces of the body below the surface layers. This is preferably done in the laboratory (see para. 18.2).

No.	Date	Species	No. of specimens	Locality	Locality description	Depth		Length		Fresh weight		Age		Storage	Analyses to be performed	Analysing laboratory	Collector	Notes
						bottom	catch	total	other	whole	piece	scale	otolith					
(1)	(2)	(3)	(4)	(5)	(6)	(7)		(8)		(9)		(10)		(11)	(12)	(13)	(14)	(15)

Fig. 1 Page of notebook proposed

The information here is somewhat more extensive than that given on the labels (Fig. 2). Numbers should be given in one sequence, preferably including the year in the code, e.g., 1972-001, 1972-002, etc. Under storage, information should be given on deep freezing, formalin fixation, kind of jars or bags. Notes should be made on the plastic, glass or metal knives used for operations, method of transport to the analysing laboratories, etc. Most items require more space than given here.

18.1.3 Fishes

To get an idea of the content of toxic substances in fishes in an area, a number of specimens should be sampled, if possible 10-15 of each species of the same age (length). It is an advantage if more than one year class can be sampled (for investigation of accumulation with age). The chosen species should be as stationary as possible, at least their yearly migration patterns should be known.

Code numbers of the fishes and relevant information about the locality, way of catching and treatment, as well as the purpose of the investigation should be noted in a special book (Fig. 1). If possible, the age should be determined directly after sampling by counting the zones of the scales and/or the otoliths, but this is often impossible, the alternative being to catch specimens of the same length. The length can be measured either as total length, fork length or standard length. Total length is recommended for fresh material.

Each fish is washed in fresh water and put into a plastic bag from which the air is pressed out by rolling the fish and the bag. This is put into another plastic bag and a label (Fig. 2) containing the most important information from the notebook is placed in

No.

Date

Species

Number of specimens

Locality

Locality
description

Depth
bottom
catch

Length
total (of the animal)
other

Weight
fresh (of the sample)

Notes

Fig. 2 Type of label to be used

The information given should be equivalent to that in the notebook. It is important that good paper is used (preferably carton paper).

the outer bag which is then tightly closed, for instance, with a rubber band, so that the writing on the label is kept dry. All double plastic bags containing fish of the same species and age and from the same locality are put into a bigger plastic bag or box which is labelled and deep frozen.

Plastic bags can contain toxic substances which might contaminate the skin of the fish. Therefore, the piece of the specimen taken out for analysis should always be taken from under the skin. To avoid the risk of contamination the fishes can also be packed in aluminium foil instead of plastic bags.

Sampling (cont.)

It is always advisable to perform the analysis as soon as possible but, if the material is kept deep frozen all the time, it seems possible to store it for several years. When reporting the results of the analyses, the storage time should be given.

8.7.1 Mussels

Since most mussel species are stationary, they ought to be excellent indicators of different kinds of pollution. But there are drawbacks: (i) most species are relatively small, which means that a large portion of their bodies must be used for the analysis; (ii) mussels are filtering organisms, which implies that a lot of particles fasten onto their gills; if such particles contaminate the samples for analysis, they may cause errors in the results; (iii) the water contained inside the shell makes fresh weight determinations difficult, especially when considering the weights of whole mussels; before deep freezing, as much as possible of this water should be removed.

When the shells are opened, to wash particles from inside and to pour off the water, the mussels may be contaminated by metals or other substances from the device used (e.g., a knife). A metal knife should be used for mussels taken for the examination of organochlorine, and a plastic knife for mussels taken for metal examination. The kind of device used should be noted in the book.

After the water has been removed, the mussels are treated as described for fishes. To avoid contamination of the soft parts by plastic bags the shells should be closed when the mussels are put into the bags. If possible, 25–50 mussels or even 100 of each species and size group from each locality should be sampled, but often one has to be satisfied with a smaller number. Specimens of the same size group and locality can be stored in the same bag.

For treatment of deep frozen samples in the laboratory, see para. 18.2, and for analyses of organochlorines and metals see Chapters 21 and 23.

8.7.2 Storage in formalin

Deep freezing of the sampled animals is the best way to store them prior to preparation for analysis, but deep freezing facilities are not always available, or if they are, transport distances are too long to keep the material deep frozen. In such cases, 7 percent neutral formalin may be needed as a substitute, but there might be a degradation of DDT to DDD and DDE (French and Jefferies, 1971). The formalin should be kept in dark glass jars with plastic (propylene) lids. The lid can be insulated by aluminium foil for material for the determination of organochlorines.

The field sampling is performed as described above. Small animals can be put straight into the jars (mussels must be opened to make it possible for the formalin to preserve the tissues). Bigger animals are more difficult to store as they are, especially if the material has to be transported to another laboratory for further treatment. In this case small pieces of the tissues to be analysed are cut out; in the case of fishes, always from under the skin and under the subcutaneous fat and red muscle layer. The weight of the piece should not be below 10 g (preferably around 15 g). When cutting out the pieces, a glass knife should be used when analysing for metals, and a metal knife when examining for organochlorines.

All data should be noted as described for fishes. The fresh weight of the piece stored in formalin must also be noted. Each glass jar containing a sample in formalin should be put, together with a label outside the jar, in a plastic bag, which is sealed.

When storing only pieces from animals in formalin, it is recommended that the investigator takes duplicate samples.

The analysis should be carried out only at laboratories acquainted with the techniques.

18.2 Laboratory treatment of biological samples for determination of toxic substances

18.2.1 Instruments for laboratory operation

Samples for mercury and organochlorine analysis should be handled with instruments of stainless steel. For other metals, broken pieces of glass with sharp edges should be used.

Containers for tissues should be made of glass. If plastic containers are used for storing materials to be analysed for organochlorines, the tissue ought to be isolated from the walls of the container by aluminium foil.

All instruments should initially be washed in detergents, to remove tissue residues. Instruments used for the preparation of samples for analysis of heavy metals should be rinsed in diluted nitric acid (HNO_3), the concentration of acid to water being 1:6; and for analysis of organochlorines such as DDT, PCB, etc., a solution of dichloromethane ($C_2H_2Cl_2$) and acetone (CH_3OCH_3), in the concentrations 1:1. All chemicals used should be of pro analisi quality.

18.2.2 Collection of material

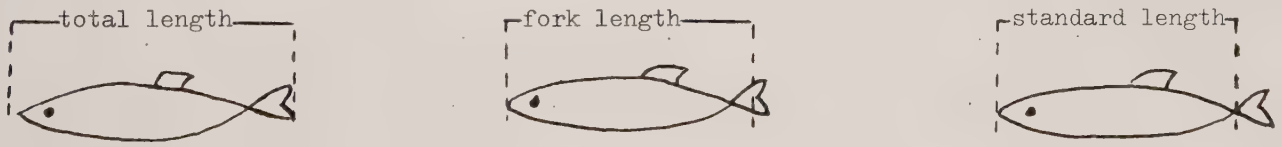
All material collected in the field should be correctly dated and determined geographically. This means that data on sampling time, water depth and geographical location should be noted as correctly as possible (see also para. 2.4.1). The biomass and the chemical conditions of the water in the sampling area should be estimated.

When tissue samples from organisms are to be analysed, the specimens should be wrapped separately in plastic bags. When entire organisms, such as small invertebrates and fishes, are to be analysed, put them in glass vessels.

All material should be deep frozen immediately at a temperature below 18° C, to prevent decomposition of tissues. The sample should be brought to the laboratory in a frozen condition.

18.2.3 Preparation of samples

Biological parameters. The sex, length, weight and if possible age of the organisms to be studied should be determined. For fish, the length can be expressed as total length, fork length or standard length (see Fig. below).



Since the length is mostly determined after a period in frozen condition, the caudal fins will often be broken. Thus it seems most feasible to use the standard length (length between the nose and last vertebra).

18.2.4 Treatment of samples for analysis

When analysing biological samples, the choice of tissues is determined by the aim of the analysis. Muscle tissue is the best material if a general indication of the organochlorine and mercury content in a population is desired. If, on the other hand, mortality within a population is suspected to be a result of the toxicity of organochlorines or mercury, the best material is tissue from the brain and liver. For analysis of other compounds, such as other heavy metals, liver and kidney are good test material. Only the preparation methods will be described here. The samples for analysis should be prepared from frozen material. If the material is fresh, there is a risk that body fluids and oils will be squeezed out from the tissue during preparation.

Treat biol 2

Vertebrates. For mammals and birds, the samples should be taken deep enough in the muscle tissue to eliminate subcutaneous fat.

For fish, the muscle sample should be taken under the red lateral muscle in order to obtain only clean fish muscle.

It is important that in all investigations the same type of muscle is chosenfor all specimens of the same species; this is because of the differences in levels within different parts of the body.

Invertebrates. If possible, one type of tissue should be used as test material for invertebrates but often the animals are too small to allow good preparation of specific tissues, and therefore homogenates of the whole body are normally used. For small fishes and invertebrates one single specimen may be too small as material for one analysis. Homogenates of several entire specimens can also be used here. If tissues samples from several specimens are to be homogenized it is important to take the same weight of tissue from the individual specimens for the homogenate.

18.2.5 Homogenization

If the homogenate is to be analysed for mercury or organochlorines a well cleaned homogenizer of stainless steel can be used. When only parts of the homogenate are sent for analysis, it is very important that the samples are taken immediately after homogenization, thus eliminating sedimentation processes in the homogenate.

Samples intended for the analysis of other metals can be freeze dried and then homogenized in a mortar made of glass. Homogenizers made of metals can rebase chips of the metal into the sample taken for analysis. This is particularly a risk when homogenizing organisms containing granules of sand, such as bottom-feeding invertebrates.

18.2.6 Determination of the water content in biological material

In some organisms, especially invertebrates, the water content may vary from time to time. Thus it is important to determine the water content in the sample, to be able to calculate the levels of pesticides, etc., found on a dry weight basis.

The following material is needed: weighing glass with a ground stopper, desiccator, incubator, and an analytical balance.

Treatment. A clean weighing glass with a ground stopper is placed into the incubator (110° C), using a pair of tongs. It is important to use the tongs every time the glass is touched, to avoid fingerprints and dirt particles on the weighing glass. The stopper is removed and the two pieces of glass are kept in the incubator for 2 h. After that the stopper is replaced and the glass (with the stopper) is put into the desiccator to cool. The empty glass and stopper are then carefully weighed on the analytical balance. The obtained weight is the weight of the dried, empty weighing glass and stopper (weight a). The next step is to remove the stopper and put about 1-2 g of the material to be studied for its water content into the weighing glass and replace the stopper. The weight is then carefully determined and the obtained weight is the fresh weight of the tissue + the dried weight of the glass and stopper (weight b). The glass is then placed in the incubator (110° C) and the stopper removed and placed within the incubator. The glass should then be incubated for at least 16 h. After that, the stopper is replaced and the glass put in the desiccator to cool, and then carefully weighed.

It should be stressed that different types of tissues and organisms have different drying times, so it is necessary to determine the drying time for every new type of material. This is done by repeated weighings after several heating spells in the incubator. When a stable weight is obtained the water has disappeared. The final weight is the weight of the dried tissue and the dried weighing glass with the stopper (weight c).

Calculations. The following calculation should be made:

Weight b - Weight a = The amount of fresh tissue (weight d)
Weight b - Weight c = The amount of water (weight e)

$$\text{percent } H_2O = \frac{\text{Weight e} \cdot 100}{\text{Weight d}}$$

To calculate the levels of the persistent compounds found in fresh tissues, on dry weight basis, the following calculations are made:

L_{ft} = Levels of persistent compounds in fresh tissue

L_{dt} = Levels of persistent compounds in dry tissue

$$100 - \%H_2O = \% \text{ dry tissue (\% DT)}$$

$$\frac{100}{\%DT} \cdot L_{ft} = L_{dt}$$

18.3 References

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Olsson, M., A.G. Johnels and R. Vaz, 1974 DDT and PCB levels in seals along the east and west coasts of Sweden. Publ.Natl.Swed.Environ.Protect.Board, (PM 420): 28 p.

Para. 18.1 has been contributed by B.I. Bybern, and para. 18.2 has been prepared by M. Olsson.

Digest 1

19. DIGESTION METHODS FOR THE DETERMINATION OF THE TOTAL CONTENT OF HEAVY METALS

The two methods described below are the standard procedures used at the Research Laboratory of the National Swedish Environment Protection Board.

As the content of heavy metals in many samples is very low, it is of great importance that pure chemicals are used, so that no heavy metals are introduced into the samples via the reagents. Several manufacturers of chemicals supply special qualities for the analysis of heavy metals. If such chemicals are not available, pro analisi quality should be used. In any case, a blank analysis, i.e., with the chemicals only, to check their possible influence on the analysis, is recommended.

19.1 A digestion method for water and sediment samples

19.1.1 Reagent

Acid solution. Mix 3 volumes of concentrated hydrochloric acid, HCl (sp.gr.=1.19) and 1 volume of concentrated nitric acid, HNO_3 (sp.gr.=1.40).

19.1.2 Procedure

Take 40 ml of the water sample, or about 1 g of the sediment sample, and add 10 ml of the acid solution. Immerse the sample in a water bath (90°C) for 30 minutes. If the sample is a sediment, dilute to 50 ml with distilled water that is free of heavy metals.

19.2 A digestion method for samples of sediments and biological material

19.2.1 Reagents

Sodium chloride solution. Dissolve 30 g NaCl in metal-free distilled water and dilute to 100 millilitres.

Acid solution. Mix carefully 10 volumes of concentrated perchloric acid, $HClO_4$ (sp.gr.=1.67) and 2 volumes of concentrated nitric acid, HNO_3 (sp.gr.=1.40).

Hydroxylammonium chloride solution. Dissolve 50 g $HONH_3Cl$ in metal-free distilled water and dilute to 100 millilitres.

19.2.2 Procedure

The sample is treated with sodium chloride and a mixture of perchloric acid and nitric acid. The free chlorine, which is developed, loosens the chemical bonds in the easiest volatilized organic mercury compounds. Gentle heating for 12 h destroys the organic material and transfers the metals into solution. Hydroxylammonium chloride is added to eliminate the free chlorine and other oxidizing substances.

Weigh in a 50-ml test tube 0.5-1 g material (dry weight) or 1-5 g (wet weight). Add 3 drops of the sodium chloride solution and 8 ml of the acid solution. Heat at 70°C for 12 h in a water bath or other suitable heating equipment. Add 3 drops of the hydroxylammonium chloride solution. Mix and dilute to 50 ml with metal-free distilled water, and then analyse.

Chapter 19 has been contributed by C.G. Johansson.

20. METHODS FOR THE CHEMICAL ANALYSIS OF SEDIMENTS

20.1 Determination of the oxidizable matter (organic carbon) by the chromic acid method

20.1.1 Introduction

This method (Walkley and Black, 1934) excludes (to 90-95 percent) the elementary carbon present as graphite and charcoal, and will therefore give the concentration of the organic matter in the sediment. Proteins may also remain unoxidized. Since carbon only constitutes about 58 percent of the soft organic remains in sediments, the carbon content can be converted to the organic matter content by multiplying the former by 1.7.

The oxidation of carbon by chromic acid may be represented as follows:

$$2\ Cr_2O_7^{2-} + 3\ C + 28\ H^+ \longrightarrow 4\ Cr^{3+} + 3\ C^{4+} + 14\ H_2O$$

and the reduction of $Cr_2O_7^{2-}$ by ferrous solution may be given as

$$Cr_2O_7^{2-} + 6\ Fe(NH_4)_2^{4+} + 14\ H^+ \longrightarrow 2\ Cr^{3+} + 6\ Fe(NH_4)^{4+} + 6\ NH_4^+ + 7\ H_2O$$

20.1.2 Reagents

All reagents should be of pro analisi quality.

Phosporic acid, H_3PO_4, concentrated, 85 percent (sp.gr. 1.71).

Sulphuric acid, H_2SO_4, concentrated, minimum 96 percent (sp.gr. 1.84).

Sodium fluoride, NaF, solid.

Potassium dichromate solution, 1 N. Dissolve 49.04 g of $K_2Cr_2O_7$ in distilled water and dilute to 1 000 ml.

Diphenylamine indicator. Dissolve carefully 0.5 g of diphenylamine in a mixture of 20 ml distilled water and 100 ml concentrated sulphuric acid.

Ferrous solution, 0.4 N. Dissolve 156.86 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in about 800 ml distilled water, add 14 ml concentrated sulphuric acid and dilute to 1 000 ml.

20.1.3 Equipment

Conical flasks, 500 ml capacity.

Burette, 25 ml capacity; scale division 0.1 ml or more.

20.1.4 Procedure

The sediment sample is either freeze dried or dried in an oven (110°C for 24 h). The dried sample is ground in an agate mortar and then passed through a 0.2 mm (80 meshes per inch) non-ferrous sieve.

A carefully weighed amount of the powdered sample (containing about 10-25 mg carbon) is placed in a 500 ml conical flask. With a pipette, add exactly 10 ml of the 1 N dichromate solution and mix carefully by swirling. Then add 20 ml of concentrated sulphuric acid carefully and mix by gently rotating the flask for 1 min. Let the mixture react for 20 to 30 min.

Sedm organ 2/metal 1

Dilute the sample to 200 ml with distilled water and add 10 ml of concentrated phosphoric acid, 0.2 g of sodium fluoride and 1 ml of diphenylamine indicator.

The sample is now back titrated with the 0.4 N ferrous ammonium sulphate solution. In the beginning the colour of the sample is dull green because of the chromous ions, and then becomes a turbid blue as the titration proceeds. At the end point, this colour changes sharply to a brilliant green, giving a one drop end point.

If more than 8 ml of the 10 ml of dichromate solution is consumed (more than 20 ml of the ferrous solution is consumed in the titration), the analysis is repeated with a smaller sediment sample.

Two-three standardization blanks (without sediment) are analysed every day in the same way.

20.1.5 Calculation

The results are calculated from the equation:

$$\text{percent C} = \frac{3.951}{g} \left(1 - \frac{T}{S}\right)$$

where

g = the sample weight in grammes
S = ml ferrous solution, standardization blank titration
T = ml ferrous solution, sample titration

A recovery of about 75 percent has been found, and this has been taken into account in the formula above.

20.2 Determination of some heavy metals by atomic absorption spectroscopy

20.2.1 Introduction

Traces of lead, zinc, copper, nickel and cobalt are determined by extraction with ammonium pyrrolidine dithiocarbamate and methyl-iso-butyl ketone, followed by vaporization of this solution in the flame of an atomic absorption spectrophotometer.

The pre-treatment and extraction procedures of this method are described here. For a general description of atomic absorption spectroscopy, see Chapter 21.

The sediment sample is freeze dried and thereafter oxidized in a low temperature asher before the extraction is begun. Low temperature ashing has proved more satisfactory than other oxidation agents.

In the low temperature ashing, the organic material is decomposed in a slow stream of oxygen, which is excited to the atomic state by a high frequency electromagnetic field. The oxidation is very powerful and efficient, although the temperature is normally as low as 100-200°C. Thus even heavy metals bound to proteins are liberated, which is not normally the case with acid digestions only. This ashing technique seems to be applicable to all metals except the very volatile mercury and its divalent compounds.

However, because of investment costs, equipment for low temperature ashing cannot be included in the standard instrumentation of every laboratory. In that case different classical chemical methods have to be used to bring the metals into solution (see Chapter 7). Because of the great number of factors governing the result, it is not possible to establish even a rough relation between the effectiveness of low temperature ashing and the classical chemical methods.

20.2.2 Reagents

All water used should be distilled (preferably three times) and taken from one and the same container. Those reagents which can be obtained in special "suprapure" qualities, specially made for analysis of trace elements (concentrated acids and some salts), should be purchased if possible. Other reagents should be of pro analisi quality.

Hydrochloric acid, 0.01 N. Dilute 8.3 ml of concentrated HCl, 37 percent (sp.gr. 1.19) to 100 ml with distilled water. 10 ml of this solution is diluted to 1 000 ml with distilled water.

Buffer solution with pH 2.4

(a) Weigh 71.628 g of disodium hydrogen phosphate, $Na_2HPO_4 \cdot 12H_2O$ and transfer it to a 1 000 ml volumetric flask, dissolve, and dilute with distilled water to the mark. This solution is 0.2 M.

(b) Weigh 21.008 g of citric acid, $C_6H_8O_7 \cdot H_2O$, and transfer it to a 1 000 ml volumetric flask, dissolve and dilute to the mark with distilled water. This solution is 0.1 M.

Mix 31 ml of solution (a) with 469 ml of solution (b). This buffer should have a pH of 2.4.

APDC solution. The complexing agent here is a 1 percent water solution of ammonium pyrrolidine dithiocarbamate (APDC). 1.00 g APDC is dissolved in 100 ml water. Transfer it to a separatory funnel and add 10 ml methyl-iso-butyl ketone (MIBK), then shake for a couple of minutes. Let the two phases separate and drain the lower layer through a filter paper. The solution must be prepared daily.

Methyl-iso-butyl ketone is used for the extraction.

Standard solutions. Standard solutions can preferably be purchased direct from the chemical suppliers. If the solutions are prepared at the laboratory, the highest quality of the different salts should be used. The solutions described below all have the concentration 1 000 mg/l if the final volume is adjusted to 1 000 ml. Before weighing, the salts must be dried to constant weight in an oven or desiccator.

Co Dissolve 4.9384 g cobolt nitrate, $Co(NO_3)_2 \cdot 6H_2O$

Cu Dissolve 2.6832 g copper chloride, $CuCl_2 \cdot 2H_2O$. Add 5 ml of 0.05 N hydrochloric acid.

Ni Dissolve 4.0489 g nickel chloride, $NiCl_2 \cdot 6H_2O$

Pb Dissolve 1.8308 g lead acetate, $PbOOCCH_3 \cdot 3H_2O$. Add 1 ml of concentrated acetic acid.

Zn Dissolve 4.5506 g zinc nitrate, $Zn(NO_3)_2 \cdot 6H_2O$

Each solution is diluted with distilled water to 1 000 ml. The solutions are stable for six months. Dilute working standards with the concentration 1 µg/ml must be prepared daily.

20.2.3 Equipment

- Spoons or spatulas of plastic material
- Parafilm or other plastic films for covering beakers
- pH meter with electrodes to check the pH of buffers and samples
- Filter paper, Whatman 42, Munktell OOH, or equivalent
- Separatory funnels, 125 ml capacity
- Centrifuge (for 3 500 rpm) with tubes of 10-15 ml capacity

Sodium metal 3 µg l

- Volumetric flasks, 5 ml capacity
- Pasteur pipettes
- Test tubes or conical flasks (10 ml capacity) with stoppers.

All glassware should be washed in warm soapy water, cold water, concentrated nitric acid, warm and cold water, and several times in distilled water.

20.2.4 Pre-treatment

0.5 g of finely powdered, dry sediment is oxidised in a low temperature asher for 4½ h or until the colour in the glass tube is red. Stir the sediment with a glass rod every ½ h. Transfer the sediment into a 50 ml beaker and add 5 ml 0.01 N HCl and 10 ml buffer solution. Use only plastic tools and wash the bottle after the transfer. Cover the beaker with parafilm. The sample should be leached for 48 h.

20.2.5 Procedure

Adjust the pH of the pre-treated sample to around 5 (4.8–5.2) by adding about 5 ml 0.2 M sodium phosphate solution. The pH is controlled with a pH meter. Pass the solution through a paper filter down into a 125 ml separatory funnel and wash the beaker and the filter so that the liquid volume is about 50 ml. Mark the 50 ml level on the funnel before starting the separation. The solution is now ready for the complexing agent and the extraction. Certain metal complexes are stable only for 3–5 h.

Add 5 ml APDC solution to every sample solution. Mix carefully. Add 10 ml MIBK to each separatory funnel and shake them for 3 min. The lower layer (the water phase) is run off and discarded, the upper, dark layer is transferred to a centrifuge tube. After centrifuging at 3 500 rpm for 10 min, a partial separation of the layers occurs. The upper layer is transferred with a fine Pasteur pipette to tubes with plugs.

To measure Zn it may be necessary to perform a dilution. Take out 1 ml and transfer it to a 5 ml volumetric flask and dilute with MIBK to 5 ml. Mix. Ready for analysis.

20.2.6 Calibration

The calibration is performed by analysing standard solutions as described above.

A series of standard solutions is prepared in the following way.

Make a solution of 30 ml 0.01 N HCl, 60 ml buffer solution (pH = 2.4), 50 ml 0.2 M sodium phosphate and 180 ml distilled water. Check that the pH is around 5. Put 50 ml of the solution in each of five separatory funnels. Remove 1 ml from funnel 2, 4 ml from No. 3, 6 ml from No. 4 and 10 ml from funnel 5. Then add to these funnels in turn 0, 1, 4, 6, and 10 ml of the standard solution with the concentration 1 µg/ml (same 5 µg/ml). Add 5 ml APDC and 10 ml MIBK to each funnel and continue as above. Even here, a dilution is prepared for the zinc analysis.

Measure the standard solutions together with the sample solution. The photometer readings of the standard solution are plotted against the concentrations.

20.3 Determination of mercury by flameless atomic absorption spectroscopy

20.3.1 Introduction

The following method for the determination of mercury employs a simple reduction and aeration procedure, where the volatile mercury is carried by a gas stream into the measuring cell in the photometer. Although the procedure described was worked out for a Coleman Mercury Analyser MAS-50, it should be applicable to other common atomic absorption photometers, with or without minor modifications.

Concentrations of mercury between 30 and 600 ng per sample can be determined.

20.3.2 Reagents

All water should be distilled (preferably three times) and taken from one and the same container. Those reagents which can be obtained in "suprapure" qualities, specially made for analysis of trace elements (concentrated acids and some salts), should be purchased if possible. Other reagents should be of pro analisi quality.

Aqua regia. Mix one part of concentrated nitric acid, HNO_3 (sp.gr. 1.40) with three parts of concentrated hydrochloric acid, HCl (sp.gr. 1.19). The amount prepared should be enough for all samples and blanks in the series.

Potassium permanganate, 6 percent. Dissolve 6 g $KMnO_4$ in about 90 ml water. Place the flask in warm water to speed up the dissolution. Cool and dilute to 100 ml.

Nitric acid, 5.6 N. 350 ml conc. HNO_3 (sp.gr. 1.40) is added carefully to 500 ml water and, after cooling, diluted with water to 1 000 ml.

Sulphuric acid, 18 N. 500 ml conc. H_2SO_4 (sp.gr. 1.84) is added carefully to 500 ml water. Cool.

Sulphuric acid, 0.5 N. 14 ml conc. H_2SO_4 (sp.gr. 1.84) is diluted with water to 1 000 ml.

Sulphuric acid, 1 N. 28 ml conc. H_2SO_4 (sp.gr. 1.84) is diluted with water to 1 000 ml.

Stannous chloride, 10 percent. Weigh 10 g $SnCl_2$ and dissolve it in 0.5 N sulphuric acid. Dilute with the same acid to 100 ml. Must be prepared daily.

Hydroxylamine hydrochloride, 3 percent. Weigh 15 g $NH_2OH.HCl$ and dissolve in water. Dilute to 500 ml.

Standard mercury solution, 1 mg/ml. Dry $HgCl_2$ to constant weight at about 100°C (2-4 h). Dissolve 0.1354 g $HgCl_2$ in 100 ml of 1 N sulphuric acid. Prepare from this solution dilutions in the concentration range 10 µg/ml to 20 ng/ml. The diluted solutions should be prepared daily.

20.3.3 Equipment

Special instrument for mercury analysis by flameless atomic absorption, or a common atomic absorption instrument equipped with a mercury lamp and a closed system for aeration of the sample, as shown in Fig. 3, Chapter 21.

All glassware should be washed in warm water, concentrated nitric acid, water, and finally, several times in distilled water.

20.3.4 Pre-treatment

The sediment sample is preferably freeze dried prior to homogenization and analysis. If a freeze drier is not available, the sample has to be dried in an oven at as low a temperature as possible (about 40° C), because some volatile mercury compounds may evaporate to some extent.

For the same reason the sample for this analysis may not be oxidized in the low temperature asher. The only digestion procedure applicable is the one with aqua regia.

20.3.5 Procedure

Treat 2-4 g finely powdered sediment with 20 ml aqua regia in a 250 ml beaker. Treat 2 blanks in the same way. Cover the beaker with a watch glass. Heat the beaker carefully for 1 min. Cool to 25°C. Transfer the contents to a 100 ml volumetric flask. Wash and dilute to 100 ml. Mix and let the solution separate.

Sedm Hg 3/tot P 1

Transfer 25 ml of the yellow solution to an aeration bottle and dilute with water to 100 ml. Add drops of permanganate solution until the sample is faintly red (the colour should be stable for at least 1 minute). Add 2.5 ml of the 5.6 N nitric acid, 2.5 ml of the 18 N sulphuric acid and 1 ml of the hydroxylamine solution. The sample should be clear and nearly colourless after the last addition (the colour should not be stable for more than about 15 sec).

Check that the photometer reading is zero, add 2.5 ml of the stannous chloride solution to the sample in the aeration bottle, close the bottle immediately and read the absorbance on the photometer.

To check the performance of the aeration system and the photometer, every fifth sample should be a standard.

20.3.6 Calibration

A calibration graph is obtained by analysing (digestion not necessary) 100 ml distilled water and some standard solutions (5-6 standards in the range 0-300 ng Hg). If the reference (distilled water without reagents) does not give zero absorbance, this value is added to those of the standard samples. A graph is constructed with absorbance versus ng Hg (absolute amount) in the aeration bottle.

20.3.7 Calculation

The results are calculated from the equation:

$$\text{ng Hg/g} = \frac{100.a}{b.c}$$

where

a = value obtained from the curve, in ng Hg (absolute amount in the aeration bottle)
b = sample weight in g
c = ml solution transferred to the Bed Bottle (e.g., 25 ml).

20.4 Determination of total phosphorus

20.4.1 Introduction

The sample is treated with concentrated nitric acid and concentrated perchloric acid in order to release the organic phosphorus. An acid ammonium molybdate reagent is added to a suitable volume of the filtered, digested sample. The resulting phosphomolybdate complex is reduced to an intense blue by ascorbic acid; the intensity of the colour is measured in a spectrophotometer at 882 nm. Samples with a phosphorus concentration ranging between 20 and 1 500 µg/g can be measured.

20.4.2 Reagents

All reagents should be of pro analisi quality.

Concentrated nitric acid, HNO_3, (sp.gr. 1.40), 65 percent.

Concentrated perchloric acid, $HClO_4$, 70 percent (sp.gr. 1.67).

Sulphuric acid, diluted, 50 ml concentrated H_2SO_4, 98 percent (sp.gr. 1.84) is diluted with distilled water to 1 000 ml.

Sulphuric acid, 9.1 N. Pour into a suitable volume (600 ml) of distilled water 253 ml concentrated sulphuric acid (sp.gr. 1.84) under cooling and continuous mixing. After cooling, dilute to 1 000 ml with distilled water.

Ammonium heptamolybdate solution, 0.073 M. Dissolve 9 g of the salt $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in distilled water and dilute to 100 ml. A new salt solution must be made if any precipitation occurs.

Potassium antimonyltartrate solution, 0.1 M. Dissolve in distilled water 3.35 g $K(SbO)C_4H_4O_6$ and dilute to 100 ml. A new solution should be made if any precipitation occurs.

Acid - molybdate reagent. Mix 200 ml of the 9.1 N sulphuric acid under continuous stirring, with 45 ml of the molybdate solution. Add 5 ml of the tartrate solution. If stored cold in a dark bottle the reagent is stable for several months.

Ascorbic acid solution, 0.4 M. Dissolve 7 g ascorbic acid, $C_6H_8O_6$ in distilled water and dilute to 100 ml. If stored cold in a dark bottle, the reagent is stable for some weeks. For preservation, 0.05 g of EDTA (ethylene diamine tetra-acetic acid) and 1 ml formic acid, HCOOH, may be added.

Phosphate stock solution. Dissolve 0.4392 g of potassium dihydrogen phosphate, KH_2PO_4 (dried to constant weight at about 105°C) in distilled water. Add 1 ml of the 9.1 N sulphuric acid and some drops of chloroform for preservation and dilute to 1 000 ml. This solution contains 100 µg of phosphate phosphorus, PO_4-P per ml.

20.4.3 Equipment

- Heating unit for Kjeldahl flasks
- Kjeldahl flasks, 100 ml capacity
- Volumetric flasks, 250 ml capacity
- Stoppered measuring cylinders, 50 ml capacity
- Spectrophotometer or filter photometer with cells for use at 882 nm.

All glassware should be washed in warm water, in dilute sulphuric acid (e.g., 5 N), in water, and finally in distilled water. If synthetic detergents are used, never use the powdered ones as these contain enormous amounts of phosphorus. Before a detergent is used, it should be checked for phosphorus.

20.4.4 Pre-treatment

The sediment sample is preferably freeze dried and then finely powdered in an agate mortar. If a freeze drier is not available, the sediment is dried in an oven at 110° C for 24 h and then powdered in the mortar.

20.4.5 Procedure

Weigh 0.5 g finely powdered sediment and trasfer it to a 100 ml Kjeldahl flask. Wash down sediment residues from the neck of the flask with as little water as possible. Moisten the sample until it has the consistency of a thin cream. Add 3 small acid-washed glass beads, 2 ml concentrated HNO_3 and 2 ml concentrated $HClO_4$. Place the flask on a pre-heated hot plate and heat slowly until dry. Let the flask cool, add 1 ml concentrated $HClO_4$ and heat again until dry. The flask must be rotated several times during the heating. Cool the flask and then add 21 ml diluted H_2SO_4 solution. Boil slowly for 10 min. Cool.

The solution is filtered (using moistened filter paper, e.g., Whatman 42), into a 250 ml volumetric flask. Wash the flask and filter and dilute to 250 ml.

Pipette 5 ml of the solution into a 50 ml graduated cylinder and add 25 ml water together with 5 drops of ascorbic acid and 1 ml sulphuric acid - ammonium molybdate solution. Mix the solution by swirling after every addition. After 5 minutes, measure the absorbance at 882 nm in a 1 cm cell against distilled water.

Sedm tot P 3/Kjeld-N 1

Three-four standard sediment samples, two of them with addition of the phosphorus standard, are always run together with the samples being analysed. A standard sediment without ascorbic acid can be used as a blank. The absorbance of this blank is subtracted from those of the samples.

20.4.6 Calibration

Mix 2-3 sediment samples (well powdered) with a known concentration of about 700-750 µg total P/g. Weigh 7 samples (0.5 g each) in 100 ml Kjeldahl flasks. Add to 5 of these flasks varying amounts of the phosphorus standard solution: 0 ml to flasks 1 and 2 (blank samples), 2 ml to flasks 3, 3ml to flask 4, 4 ml to flask 5, 5 ml to flask 6, and 6 ml to flask 7.

The absorbance is plotted against the concentration in µg/0.5 g sample. The readings for the blank sediment samples are deducted from those with the phosphorus standard solution added.

20.4.7 Calculation

The results are calculated from the equation:

$$X = \frac{a \cdot 1}{b}$$

X = total concentration of phosphorus in µg/g
a = value obtained from the curve, in µg/0.5 g
b = sample weight in g

20.5 Determination of Kjeldahl nitrogen

20.5.1 Introduction

Kjeldahl nitrogen is the sum of organic nitrogen (org-N) and ammonia nitrogen (NH_3-N). With the method described here, concentrations ranging from 0.05 to 0.75 percent (0.4 and 7.5 mg/g) of ammonia nitrogen, NH_3-N, can be determined when a 1 cm cell is used for the photometric measurement. The nitrogen compounds are converted to ammonium sulphate. The ammonia is released and distilled off from this salt (in order to eliminate the disturbing substances) and determined as indophenol blue according to the ammonia method (para. 16.8.1).

20.5.2 Reagents

All water should be distilled and taken from one and the same container. All reagents used should be of pro analisi quality except for the sodium hypochlorite.

Concentrated sulphuric acid, H_2SO_4, 98 percent (sp.gr. 1.84).

Copper sulphate solution, 0.1 M. Dissolve 2.5 g of $CuSO_4 \cdot 5H_2O$ in distilled water and dilute to 100 ml.

Sodium hydroxide solution, 10 N. Dissolve carefully 400 g NaOH in about 400 ml of distilled water. Cool to room temperature and dilute to 1 000 ml.

Sulphuric acid, 0.1 M. Add carefully 5.6 ml concentrated H_2SO_4 to 900 ml water. Cool and dilute to 1 l.

Manganous sulphate solution, 0.625 mg Mn/l. Dissolve 0.1923 g $MnSO_4 \cdot H_2O$ in water and dilute to 1 l. Dilute 10 ml of this solution to 1 l.

Acetone

Methanol

Sodium phenate solution

(a) Dissolve 62.5 g phenol C_6H_5OH, in 20 ml acetone and dilute to 100 ml with methanol. Handle the phenol carefully; it is toxic and may cause skin irritation.

(b) Dissolve 27.5 g sodium hydroxide NaOH, in water and dilute to 100 ml. Mix 1 part of (a), 1 part of (b) and 2 parts of water immediately before use.

Sodium hypochlorite solution (Variant I), 0.9 g active chlorine in 100 ml. The commercially available sodium hypochlorite solution must be stored in dark and cool. Dilute to 250 ml with distilled water, 10 ml of the sodium hypochlorite solution, which usually contains 8-12 percent active chlorine. However, the active chlorine content changes with time and must be determined every time a new hypochlorite reagent is prepared (at least weekly). Pipette 10 ml of this solution into a titration flask containing 25 ml distilled water, 5 ml concentrated acetic acid and 1 g potassium iodide. Titrate against 0.1 M sodium thiosulphate (24.82 g $Na_2S_2O_3 \cdot 5H_2O$ in 1 l water) with a few drops of starch indicator. Calculate the active chlorine content from the expression:

$$x = \frac{n \cdot a \cdot 35 \cdot 5 \cdot 25}{b}$$

where

x = mg active chlorine per ml of the hypochlorite solution
n = the exact concentration of sodium thiosulphate in mole/l
a = ml sodium thiosulphate solution consumed in the titration
b = volume, ml, of the hypochlorite solution

Calculate the volume of the sodium hypochlorite solution which contains 0.9 g active chlorine. Add 1 ml of the manganous sulphate solution and dilute to 100 ml. Store the solution dark and cool and prepare it weekly.

Sodium hypochlorite solution (Variant II). Dissolve 2.5 g of Trion (common name for 1,3-dichlorcyanuric acid) in ammonia-free water. Add 1 ml of the manganous sulphate solution and dilute to 100 ml. Store the solution cold and prepare it weekly.

Ammonium standard solution. Weigh 0.2357 g of ammonium sulphate, $(NH_4)_2SO_4$ (dried to constant weight at about 105° C), dissolve in distilled water and dilute to 1 000 ml. This solution contains 0.05 mg/ml of ammonia-nitrogen, NH_3-N.

20.5.3 Equipment

- Heating unit for Kjeldahl flasks
- Kjeldahl flasks with ground glass fitting KS 29, 100 ml capacity
- Glass apparatus for distillation as in Fig. 13 in Chapter 16 (page 148)
- Volumetric flasks, 25 ml capacity
- Spectrophotometer or filter photometer with cells for use at 625 nm.

20.5.4 Sample pre-treatment

The sediment sample is preferably freeze dried and then finely powdered in an agate mortar. If a freeze drier is not available, the sediment is dried in an oven at 110° C for 24 h and then powdered in the mortar.

Sedm Kjeld-N 3

20.5.5 Procedure

Digestion in H_2SO_4. Put 0.1 finely powdered sediment into a Kjeldahl flask and add 15 ml water. The same volume of water can be used for the blank samples, and they should be run on the same day as the sediment samples.

Add 3 small glass beads, 4 ml concentrated H_2SO_4, and 1 ml of 0.1 M $CuSO_4$ solution. Place the flask in a heating unit and increase the heat gradually. After the colour becomes light green and no white smoke is noticeable, the heating is continued for 30 min. Cool and add carefully 25 ml water. Continue cooling.

Distillation. Add 20 ml 10 N NaOH by tilting the flask so that the hydroxide can be injected below the sample solution surface. Assemble the distillation apparatus immediately, with a 25 ml volumetric flask containing 1 ml 0.1 M H_2SO_4, as the receiver; place the former carefully on the heating stand and the receiver in a beaker with iced water. The process should be continued for 5 min after the gas has collected in the receiver (it creates a rattling sound). Remove the receiver carefully in order to avoid back flow and wash the tip with water. Cool the receiver and dilute to 25 ml.

Pipette 2 ml of the solution into a 25 ml volumetric flask, add 2 ml of the sodium phenolate solution and 2 ml of the sodium hypochlorite reagent (swirl the flask between every addition). Dilute to 25 ml and measure the absorbance after 45 min at 625 nm, in a 1 cm cell, against distilled water. The colour is stable for 24 h.

The absorbance of the blank is subtracted from those of the samples.

20.5.6 Calibration

Pipette 2, 5, 10 and 15 ml of ammonium standard solution into separate Kjeldahl flasks and add water to bring the volume of each to 15 ml. These solutions correspond to the concentrations 0.008 mg, 0.020 mg, 0.040 mg and 0.060 mg/25 ml.

Fifteen millilitres of distilled water is used as blank sample. The standard samples and the blanks are analysed as described above.

The absorbance of the blank is subtracted from those of the standards, and the corrected absorbances are plotted versus the ammonia concentration as mg NH_3-N/25 ml.

20.5.7 Calculation

The results are calculated from the equation:

$$X = \frac{a \cdot 25 \cdot 100}{2 \cdot b}$$

where

X = % NH_3-N
a = mg NH_3-N/25 ml, obtained from the standard curve
b = amount of sample, mg
25/2 = dilution factor

To convert percent NH_3-N to percent NH_3, the result is multiplied by the factor 1.214.

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Chapter 20 has been compiled by E. Olausson.

Gen AA 1

21. METAL ANALYSIS BY ATOMIC ABSORPTION AND FLAME EMISSION TECHNIQUES

Analysis of metals by atomic absorption and flame emission techniques may be regarded as consisting of two different stages; the pre-treatment or digestion and the instrumental analysis. The digestion techniques were described in Chapter 19. The instrumental analysis seldom involves chemical steps, such as the use of reagents, but is in most cases a purely instrumental operation. Thus reference should be made to the manufacturer's instructions.

In this chapter questions of general interest will be discussed, such as calibration methods, enrichment procedures, etc. In addition, the determination of mercury has been included as it involves a special technique: flameless atomic absorption. The determination of sodium and potassium is included, as these elements cannot be determined by purely wet chemistry methods.

21.1 General procedure for analysis by atomic absorption

21.1.1 Introduction

Choose the correct hollow cathode lamp for the analysis and let it warm up for at least 15 min. During this period, align the instrument, position the monochromator at the correct wave length, select the proper monochromator slit width, adjust the hollow cathode current according to the manufacturer's recommendation, light the flame and regulate the flow of fuel and oxidant, adjust the burner for maximum percent absorption and stability, and balance the photometer.

Run a series of standards of the element under analysis and construct working curves by plotting the concentrations of the standards against absorbance. Aspirate the samples into the flame and determine the concentrations from the calibration curve. For best results, run standards each time a sample or series of samples are run.

When analysing metals at the "trace level" (e.g., µg/l) it is insufficient to use chemicals of common analytical grade or pro analisi qualities. These chemicals - especially the acids - contain metals in unacceptable concentrations, thus giving contributions to the samples of the same order of magnitude as the original metal content in the samples. All chemicals should therefore be of the highest possible purity (e.g., Merck "Suprapur" or BDH "Aristar"). If the acids of the ultra pure quality cannot be obtained it may be advisable to redistil the analytical grade products. Thus, concerning standards, it is both economical and less time-consuming to buy the commercially available stock solutions. As an alternative, the reagents (but not the standards, of course), may be free from metal impurities by extracting with APDC and MIBK (para. 21.1.4).

If the work is carried out at the "pollution level" (mg/l) the purity requirements may be less restrictive. In any case it is strongly recommended to perform blank analysis of all the different reagent solutions and the distilled water used, to be sure that no unacceptable metal contributions are present. The water used for the preparation of standards and dilution of samples should preferably be distilled two or three times.

21.1.2 Preparation of standards

Stock solutions can be obtained commercially. They can also be prepared from high purity metals, oxides or non-hygroscopic reagent grade salts using redistilled nitric or hydrochloric acids. The stock solutions are prepared at concentrations of 1 000 mg of the metal per litre. For exact information, follow the instrument manufacturer's operating instructions.

Standard solutions are prepared by diluting the metal stock solution at the time of analysis. For best results the diluted calibration standards should be fresh prepared each time an analysis is to be made, and discarded after use. Prepare a blank and some calibration standards within the appropriate working range. If samples are preserved with acid the acid strength of the calibration standards should be similarly adjusted. The acidity too must be adjusted to that of the digested samples (which contain much higher concentrations of acid than the preserved samples do).

21.1.3 Standard addition method for calibration

Where the sample matrix (e.g., content of salt, organic substances, etc.) is so complex that viscosity, surface tension, and components cannot be accurately matched with the standards, the method of standard addition must be used. This method relies on the addition of a small, known amount of metal to a portion of the sample - the difference in absorbance between this and the original solution gives the slope of the calibration curve. Two methods are available: single addition and multiple addition. In all cases it is necessary to ensure that the calibration is linear over the region of interest and that no non-atomic absorption is present.

Single addition. Take two aliquots from the sample. To one, add a suitable volume of the standard so that the analyte concentration is approximately 10-50 percent higher; to the other, add an equal volume of a blank solution of similar matrix (in many cases this means addition of distilled water and acid) as the standard but without the analyte. Calculate the sample concentration from:

$$\frac{C_s}{C_s + a} = \frac{A_s}{A_a}$$

where

C_s = concentration of element in sample aliquot after dilution

A_s = absorbance of sample

a = concentration of element added in diluted sample

A_a = absorbance of sample aliquot with addition

Multiple addition. Prepare multiple aliquots of sample solution. To each of these add a different amount of the element ensuring that the volumes of all solutions are identical. To one of the samples add a blank solution of similar matrix as the standard but without the analyte. Measure the absorbance of each aliquot and plot a calibration graph. Extrapolate to zero absorbance to give the concentration of the sample (Fig. 1).

21.1.4 Enrichment technique

When the concentration of the metal is not sufficiently high to be determined directly, or when considerable amounts of dissolved solids are present in the sample, certain metals can be chelated and extracted with organic solvents. One advantage of this is that unwanted bulk matrix components (such as major salts in sea water) are often not extracted. Another advantage is that when aspirating an organic solvent into an atomic absorption spectrophotometer an increase in nebulization efficiency is obtained. The sensitivity of this method is 2-5 times higher than measuring in an aqueous phase of similar concentration. Extraction is rapid, high concentration factors (20-50 x) can be achieved and many elements can be extracted simultaneously.

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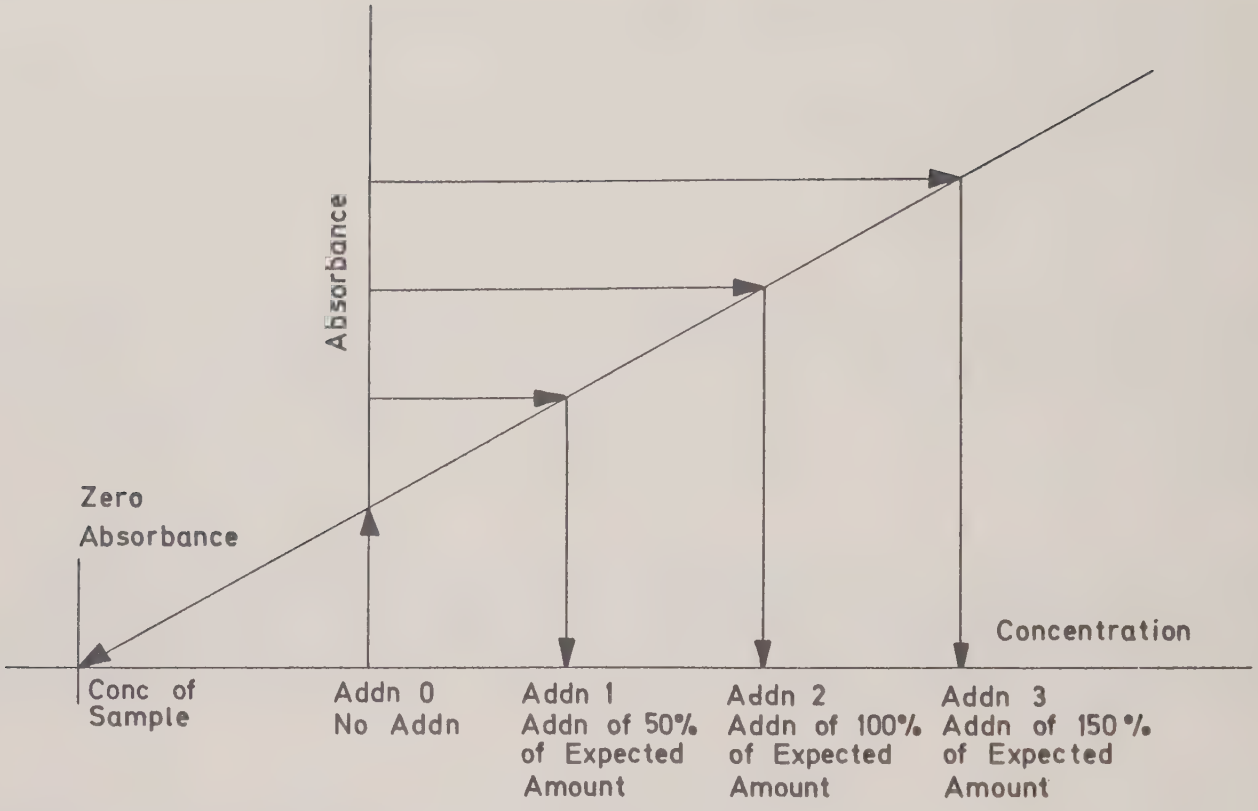

Fig. 1 Calibration graph

Metyl-iso-butyl ketone (MIBK) is the solvent most commonly used because it is suitable for combustion in normal AA (atomic absorption) flames. It is relatively involatile and thus the sample concentration will not change rapidly on standing. Cyclohexane may be added to MIBK in the ratio 1:4 to decrease the solubility of the solvent system in aqueous phase, thereby increasing the extraction efficiency.

Ammonium-pyrrolidine-dithio-carbomate (APDC) is the most popular extractant used for AA. Used with MIBK, it will extract a large number of metal over a wide pH range.

Metal	pH range	Notes
Cd	1-6	
Co	2-4	
Cr	3-9	Cr^{6+}; Cr^{3+} if the water is heated with APDC
Cu	0.1-8	
Fe	2-5	
Mn	2-4	Complex unstable
Ni	2-4	
Pb	0.1-6	
Zn	2-6	

It is assumed that the water analyst desires to extract many elements simultaneously so the method given below is general.

Conditions. As solvent use metyl-iso-butyl ketone (MIBK). For chelating use ammonium-pyrrolidine-dithio-carbomate (APDC). Extract with 1 percent APDC in water. The extractant solution has to be prepared daily. The pH should be 2.5, to be adjusted with hydrochloric acid or ammonium solution. To obtain an enrichment factor of 10, use 100 ml of sample and 10 ml MIBK.

Method. Pipette 100 ml of the sample into a beaker, adjust the pH to 2.5 and transfer the mixture to a 200 ml volumetric flask. Add 5 ml of the extractant solution and 10 ml MIBK. Shake for 2 min and allow the layers to separate and add deionized, distilled water until the MIBK layer is completely in the neck of the flask.

Aspirate the MIBK layer into the flame of the AA photometer and record the scale readings.

Standards. The standards must be in the same solvent as the sample. The best way to accomplish this is to extract the standards from an aqueous solution of similar matrix using the extraction technique employed for the samples. Many extractions are not 100 percent efficient and the extraction of standards will result in a correction for the slight inefficiences which sometimes occur.

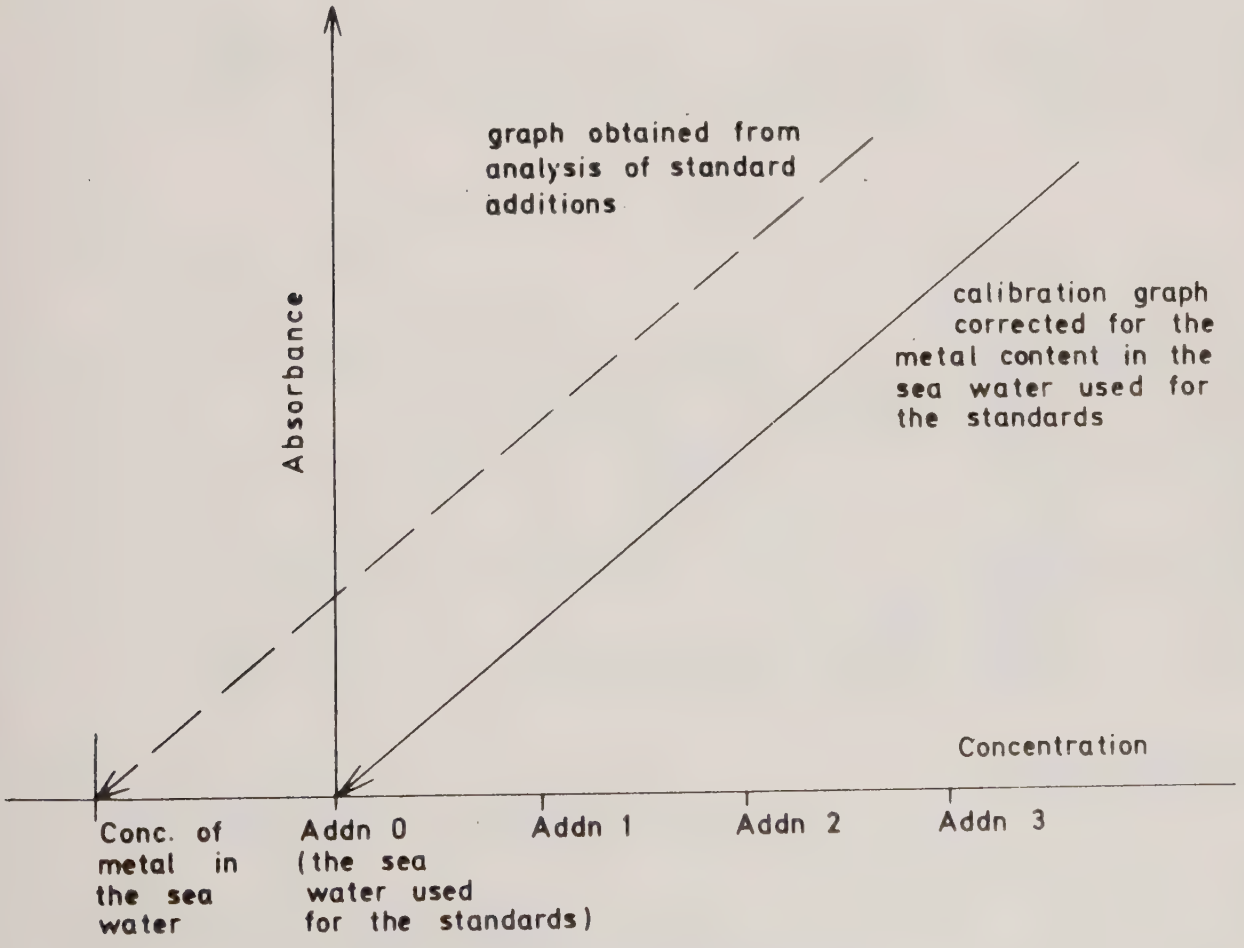
graph obtained from analysis of standard additions
calibration graph corrected for the metal content in the sea water used for the standards
Absorbance
Concentration
Conc. of metal in the sea water
Addn 0 (the sea water used for the standards)
Addn 1
Addn 2
Addn 3

Fig. 2 Corrected calibration graph

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Most inland and fresh waters can be compared with standards prepared in deionized, distilled water. Sea water must be compared with standards having the same or nearly the same salt content. In this case one of the samples or a mixture of several samples is taken and analysed carefully by using the multiple addition method. The solutions with addition are then used as standards for the samples. However, the calibration graph must be corrected for the metal content of the sea water used for the preparation of the standards (see Fig. 2).

21.2 Determination of free mercury by flameless atomic absorption

21.2.1 Introduction

Mercury in the environment often originates from natural sources such as ore bodies or volcanoes, as well as from man-made sources such as chlorine plants and fossil-fuelled power stations. In fresh water the natural level appears to be around 0.06 µg/l, and approximately 0.03 µg/l in sea water.

Mercury may be present in waters as free (ionic) mercury or as organically bound mercury.

The method described here is applicable without interference to all types of water and will give the amount of free mercury in the sample. If total mercury is to be measured, one of the digestion methods (see Chapter 19) has to be used. Although methyl mercury is volatile, it is not co-determined, as only the atomic state of the element absorbs radiation.

The flameless atomic absorption procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapor. The mercury is reduced to the elemental state and aerated from the solution in a closed system. The mercury vapour passes through a cell positioned in the light path of a spectrophotometer. Absorbance is measured as a function of mercury concentration.

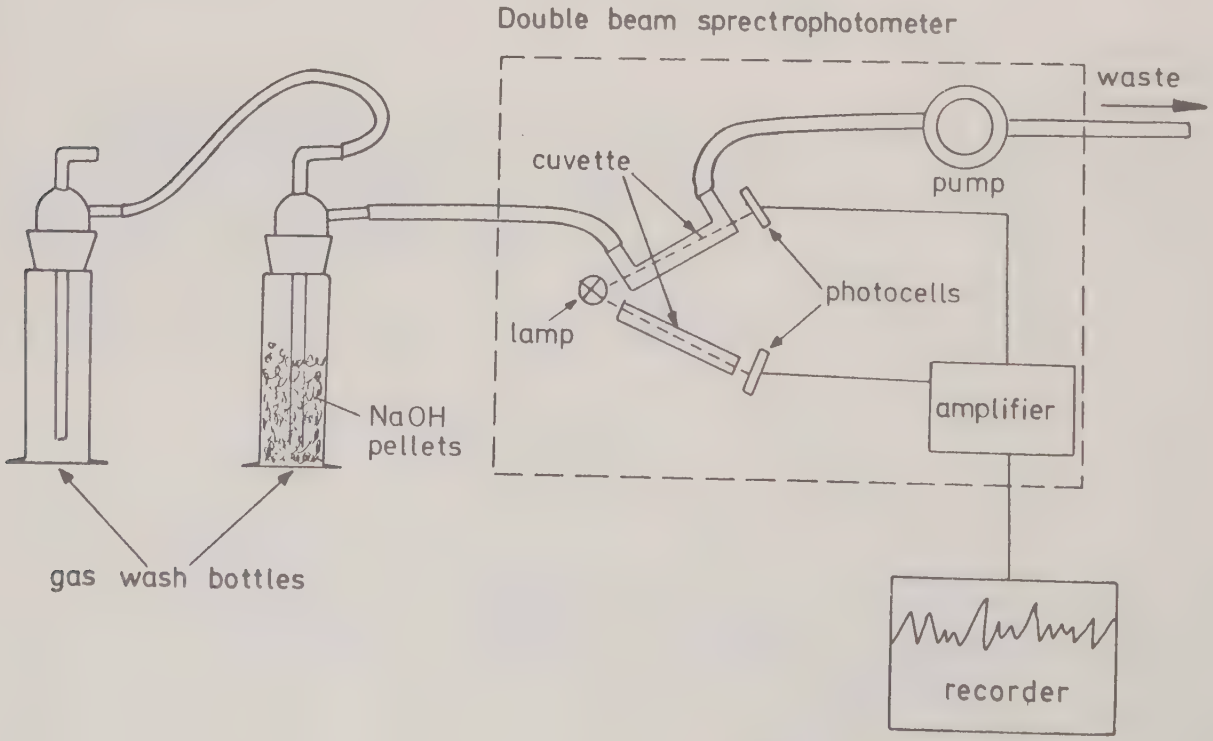

Fig. 3 Composition of equipment for flameless atomic absorption

21.2.2 Reagents

Stannous chloride ($SnCl_2$): dissolve 20 g $SnCl_2$ in 100 ml of specially purified concentrated HCl.

0.1 N hydrochloric acid (HCl): 8 ml concentrated redistilled HCl, is diluted to 1 000 ml with deionized H_2O.

Sodium hydroxide (NaOH): pellets

Hg standards: dilute 1.350 g $HgCl_2$ to 1 000 ml with 0.1 N HCl. This solution contains 1.00 mg/ml[2].

Mercury standard solution:

(1) Dissolve 1.350 g dried, mercuric chloride, $HgCl_2$, in 0.1 N HCl and dilute to 1 000 ml. This solution contains 1 mg Hg/ml.

(2) Dilute 1 ml of the concentrated solution (1) to 100 ml with 0.1 N HCl. This solution contains 10 µg Hg/ml.

(3) Dilute 1 ml of solution (2) to 100 ml with 0.1 N HCl. This solution contains 100 ng Hg/ml. It must be freshly prepared every day.

21.2.3 Equipment (see Fig. 3)

Spectrophotometer designed for measurements of mercury using the cold vapour technique (including "mercury lamp", a suitable absorption cell and air pump).

Recorder which is compatible with the spectrophotometer.

Two gas wash bottles.

21.2.4 Procedure

A sample volume of 50 ml is normal. If the mercury content of the sample is high use a smaller volume and dilute to 50 ml with 0.1 N HCl.

(1) Pipette 50 ml of the 0.1 N HCl in the first gas wash bottle.

(2) Add 1 ml of the $SnCl_2$ solution and measure the absorbance. This gives the blank value.

(3) Add 5 ng (50 µl) of the mercury standard solution (3) and measure the absorbance. In the same way, add and measure 10 ng, 20 ng and 50 ng.

This gives the calibration graph for a sample volume of 50 ml. Plot the absorbance versus mercury concentration given as ng Hg/50 ml.

(4) Empty the gas wash bottle.

(5) Pipette 50 ml of the sample (or an aliquot), add 1 ml $SnCl_2$ and measure the absorbance.

(6) Add a suitable amount of the mercury standard solution to the sample and measure, in order to check the calibration graph.

(7) Analyse for the samples as outlined under (4) and (6).

Hg 3

21.2.5 Calculation

Determine the peak height of the unknown from the chart and read the mercury value from the calibration graph.

Calculate the mercury concentration in the sample from the formula:

$$\text{ng Hg/l} = \text{ng Hg in aliquot} \cdot \frac{1\,000}{\text{volume of aliquot}}$$

21.3 Determination of sodium and potassium with the flame emission technique

21.3.1 Introduction

Sodium and potassium are present in all natural waters. The determination of sodium and potassium with a flame photometer or an atomic absorption photometer is a rapid, sensitive and accurate method.

In practical routine work sodium and potassium have to be analysed by these rather sophisticated instrumental methods, as no titrimetric or absorptometric (colorimetric) methods are available.

The solution to be analysed is sprayed into a gas flame and the excitation is carried out under carefully controlled and reproducible conditions. A monochromatic device is used to isolate the desired spectral line (589 nm for sodium and 768 nm for potassium) and a photo tube potentiometer circuit measures the intensity of the light. The light emitted is approximately proportional to the concentration of the element.

21.3.2 Reagents

Sodium-standard solution (NaCl). Dissolve 2.5422 g NaCl (analytical grade, dried in an oven at 105° C for about 1 h) in distilled H_2O and dilute to 1 000 ml. This gives a concentration of 1 000 mg Na/l.

Working standard solution. Dilute 2 ml of the standard solution above to 100 ml with distilled H_2O, which gives a concentration of 20 mg Na/l.

Potassium standard solution. Dissolve 1.9068 g KCl (analytical grade, dried in an oven at 105° C for about 1 h) in distilled water and dilute to 1 000 ml. This gives a concentration of 1 000 mg K/l.

Working standard solution. Dilute 1 ml of the standard solution above to 100 ml with distilled H_2O, which gives a concentration of 10 mg K/l.

NOTE: All standards should be kept in polyethene bottles to avoid contamination from glass

21.3.3 Equipment

- Flame photometer, or atomic absorption photometer.

21.3.4 Procedure, calibration and calculation

These steps of the analysis are carried out in the same way as described for atomic absorption photometry.

21.4 References

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This chapter has been contributed by Ö. Lindgren (General Introduction and para. 21.1.4) and C.G. Johansson.

Al 1

22. METHODS FOR THE COLORIMETRIC DETERMINATION OF ALUMINIUM, IRON, MANGANESE AND COPPER IN NATURAL WATERS

22.1 Determination of aluminium with pyrocatechol violet (Brenzcatechin)

22.1.1 Introduction

Aluminium is present in practically all natural water supplies as a soluble salt, a colloid, or an insoluble compound. All fractions may appear in treated water as a residue from alum coagulation.

This method is intended for low level determination of aluminium in fresh and saline waters, especially in settled and filtered waters from treatment plants. Negative errors are caused by fluoride (more than 0.5 ppm), chromium(III) (more than 0.05 ppm) and phosphate (more than 1.5 ppm P). Positive errors are caused by iron(II) (more than 1 ppm).

In this method, dilute aluminium solutions buffered within the pH range 6.1 to 6.2, produce, with pyrocatechol violet, a violet complex which exhibits maximum absorption at 585 nm. The optimum aluminium range extends up to 700 µg/l. In order to avoid interferences in the reaction, iron is complexed by adding 1,10-phenanthroline. Aluminium is made soluble by pre-treatment with potassium peroxodisulphate.

22.1.2 Reagents

Sulphuric acid, 4.5 M. Add 250 ml of concentrated sulphuric acid, H_2SO_4 (sp.gr. 1.84) to 500 ml distilled water. Cool to room temperature and dilute to 1 000 ml.

Aluminium standard solution, 500 mg/l. Dissolve 8.792 g dry aluminium potassium sulphate (also called potassium alum) $KAl(SO_4)_2 \cdot 12H_2O$ in distilled water and dilute to 1 000 ml in a volumetric flask.

Alternatively, dissolve 0.5000 g aluminium wire (Analar, B.D.H. or equivalent) in 20 ml concentrated hydrochloric acid (sp.gr. 1.18) and dilute to 1 000 ml with distilled water. Aluminium standards ready for use can also be purchased.

Hydroxyl ammonium chloride solution, about 14 percent. Dissolve 70 g hydroxyl ammonium chloride ($H_4Cl\,NO$) in distilled water and dilute to 500 ml. Store in a polyethene bottle.

1,10-Phenanthroline reagent solution. Dissolve 0.70 g of 1,10-phenanthroline hydrate ($C_{12}H_8N_2 \cdot H_2O$) in the hydroxyl ammonium chloride solution and dilute to 500 ml with that solution. (This solution will normally have a slight pink colour.)

Dye solution. Dissolve 0.210 g pyrocatechol violet ($C_9H_{14}O_7S$) in approximately 20 ml water, allow to stand for 5 min, mixing the solution from time to time. Dilute to 200 ml with distilled water.

Buffer solution. Dissolve 210 g hexamethylenetetraamine $(CH_2)_6N_4$, in about 300 ml distilled water, in a 500-ml volumetric flask. Add, using a suitable burette, 21 ml of concentrated ammonia solution, NH_3 (sp.gr. 0.91). Dilute, without delay, to the mark with distilled water. Check that the pH is 6.1 ± 0.1. Store the solution in a polyethylene bottle.

22.1.3 Equipment

- Spectrophotometer or filter colorimeter for use at 585 nm.

22.1.4 Sampling and sample pre-treatment

Collect the samples in polyethylene bottles with caps of the same material or in bottles of resistent glass. Preserve the samples by adding 1 ml of the sulphuric acid (4.5 M) per 100-ml sample.

Mix the sample thoroughly and transfer 50 ml into a 200-ml Erlenmeyer flask. Add 0.50 ± 0.01 g of potassium peroxodisulphate, $K_2S_2O_8$, and heat to boiling. Continue boiling until the sample volume is reduced to about 5 ml. Cool to room temperature and transfer the sample quantitatively to a 25- or 50-ml volumetric flask. Treat a blank sample of 50 ml distilled water in the same way.

Alternatively, transfer 50 ml of the sample to a bottle of resistent glass (capacity 100 ml) with a screw cap. Add 0.50 ± 0.01 g of potassium peroxodisulphate, $K_2S_2O_8$, screw on the cap and boil in an autoclave or pressure boiler at a pressure of 2 atm (temperature about 120° C) for 30 min. Cool to room temperature. Treat a blank sample of 50 ml distilled water in the same way.

If the sample contains excessive amounts of chloride (e.g., sea water), add a small amount of hydroxylammonium chloride in order to eliminate free chlorine. Let the sample stand for about one min.

22.1.5 Procedure

(1) To the sample in the volumetric flask add 1 ml of the 1,10-phenanthroline reagent solution. Mix well. Treat the blank sample in the same way.

(2) Pipette 2 ml of the dye solution into the flask and mix.

(3) Pipette 10 ml of the buffer solution into the flask, screw on the cap and mix. Dilute to the mark with distilled water. Check the pH, 6.1 ± 0.1.

(4) After 15 min measure the absorbance, with distilled water in the reference cell at 585 nm. The colour is not stable for more than 2 h.

22.1.6 Calibration

Prepare a working standard solution by pipetting 1 ml of the aluminium standard solution into a 500 ml volumetric flask. Add 10 ml of the 4.5 M sulphuric acid and dilute to the mark with distilled water. Mix well. Limited shelf life. Prepare daily.

Transfer 0 (reagent blank), 10, 20 and 30 ml of the working standard solution to 100 ml-volumetric flasks in order to prepare a series containing 0, 0.1, 0.2 and 0.3 mg aluminium/l respectively. Dilute to the mark with distilled water.

Complete the determination as outlined above. Plot absorbance against mg Al/l.

22.1.7 Calculation

Refer to the standard curve for the aluminium concentration and express the results in mg Al per litre.

22.2 Determination of iron with 2,4,6-tripyridyl-s-triazine (TPTZ)

22.2.1 Introduction

Iron is one of the most abundant metals in the earth's crust. Despite the wide distribution, natural waters contain variable, but minor amounts of iron. This is due to the fact that iron is readily precipitated as the hydroxide.

Fe 2

This method is intended for the determination of total iron, that includes ferric and ferrous iron in the ionized form, and in suspension as the colloidal hydroxide. Most probably the iron bound in the common, humic complexes is also determined.

Copper, cobalt and nickel interfere in the procedure, giving coloured complexes that absorb at 593 nm, the wave-length used in the determination. The resulting relative errors are less than 2 percent at 2.5 mg/l Cu(II), 2.4 mg/l Co(II) and 5.3 mg/l Ni(II). Many other metals can give complexes with TPTZ which is therefore added in excess. If interfering ions are known to be present or suspected, an internal standard can be used.

The minimum detectable concentration is 1 µg Fe/l with a spectrophotometer, using a 10-cm cell. Up to 1 500 µg Fe/l can be determined directly (1-cm cell). The colour intensity is independent of pH in the range 3.4 to 5.8.

Iron is made soluble by pre-treatment with potassium peroxodisulphate; it is then reduced to the ferrous state with hydroxylamine hydrochloride and treated with 2,4,6-tripyridyl-s-triazine (TPTZ). A suitable pH is obtained by use of an acetate buffer. The violet complex is measured photometrically at 593 nm.

22.2.2 Reagents

Sulphuric acid, about 4.5 M. Add 250 ml concentrated sulphuric acid, H_2SO_4 (sp.gr. 1.84) to 500 ml distilled water. Cool to room temperature and dilute to 1 000 ml.

Iron standard solution, 100 mg/l. Dissolve 0.7020 g ferrous ammonium sulphate, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (dried in a desiccator), in distilled water, add 10 ml of the sulphuric acid and dilute to 1 000 ml with distilled water. Alternatively, dissolve 0.1000 g electrolytic iron wire in 10 ml of the sulphuric acid and dilute to 1 000 ml. Iron standard solutions can also be purchased ready for use.

Hydroxylammonium chloride solution, 10 percent. Dissolve 100 g hydroxylammonium chloride (H_4ClNO) in distilled water and dilute to 1 l. If the salt contains iron, it can be removed by adding 10 ml of the TPTZ solution, 1 g of sodium perchlorate, $NaClO_4$, and extracting the solution with nitrobenzene. Store in a polyethene bottle.

Sodium acetate solution, 3 M. Dissolve 408 g sodium acetate trihydrate, $CH_3COONa \cdot 3H_2O$, in distilled water and dilute to 1 l.

TPTZ-solution, 0.001 M. Dissolve 0.312 g 2,4,6-tripyridyl-s-triazine (TPTZ) in 1 ml of concentrated hydrochloric acid and dilute to 1 l with distilled water. Store in a polyethene bottle.

22.2.3 Equipment

- Spectrophotometer or filter colorimeter for use at 593 nm.

22.2.4 Sampling and sample pre-treatment

Collect the samples in polyethylene bottles with caps of the same material or in bottles of resistent glass. Preserve the samples by adding 1 ml of the sulphuric acid (4.5 M) per 100 ml sample.

Mix the sample thoroughly and transfer 50 ml to a 200-ml Erlenmeyer flask. Add 0.50 ± 0.01 g of potassium peroxodisulphate, $K_2S_2O_8$, and heat to boiling. Continue boiling until the sample volume is reduced to about 5 ml. Cool to room temperature and transfer the sample quantitatively to a 25- or 50-ml volumetric flask. Treat a blank sample of 50 ml distilled water in the same way.

Alternatively, transfer 50 ml of the sample to a bottle of resistent glass (capacity 100 ml) with a screw cap. Add 0.50 ± 0.01 g of potassium peroxodisulphate, $K_2S_2O_8$, screw on the cap and boil in an autoclave or pressure boiler at a pressure of 2 atm (temperature about 120° C) for 30 min. Cool to room temperature. Treat a blank sample of 50 ml distilled water in the same way.

If the sample contains excessive amounts of chloride (e.g., sea water), add a small amount of hydroxylammonium chloride in order to eliminate free chlorine. Let the sample stand for approximately 1 min.

22.2.5 Procedure

(1) To the sample in the volumetric flask add 2 ml of the hydroxylammonium chloride solution, mix, then add 2 ml of the TPTZ solution. Mix again. Treat a reagent blank sample of 50 ml distilled water in the same way.

(2) Add 2 ml of the sodium acetate solution, mix, and dilute to the mark with distilled water. If a bottle is used, screw on the cap and mix.

(3) Measure the absorbance, with distilled water in the reference cell, after 5 min at 593 nm. The coloured complex is stable for at least 24 h.

22.2.6 Calibration

Prepare a working standard solution by pipetting 5 ml of the iron standard solution into a 500-ml volumetric flask. Add 5 ml of the 4.5 M sulphuric acid and dilute to the mark with distilled water. Mix well.

Transfer 0 (reagent blank), 5, 10, 20 and 40 ml of the working standard solution to 100-ml volumetric flasks in order to prepare a series containing 0, 50, 100, 200 and 400 µg iron/l respectively. Add 1 ml of the sulphuric acid and dilute to the mark with distilled water.

Complete the determination as outlined above. Plot absorbance against µg Fe/l.

22.2.7 Calculation

Refer to the standard curve for the iron concentration and express the results in µg Fe/l.

22.3 Determination of manganese with formaldoxime

22.3.1 Introduction

Manganese may exist in a soluble form in a neutral water but is readily oxidized to higher oxidation states. Manganese imparts objectionable and tenacious stains to laundry and plumbing fixtures.

This method is intended for the determination of total manganese, that is, manganese in all its possible oxidation states in an unfiltered sample. The main interferences in the procedure are Ca, Mg and Fe due to the high pH (9 to 10) at which the Mn complex is formed. The interfering metals form precipitates resulting in undesired turbidity. Iron also forms a weakly coloured formaldoxime complex with an absorption maximum near 525 nm. To control the interferences, EDTA is added which acts as a complexing agent in the presence of hydroxylammonium chloride. Iron also is added in order to completely complex the iron in the sample. (This requires a minimum concentration of iron in the sample.)

Phosphate also interferes when present in concentrations above 5 mg PO_4 per litre. The amounts of cobalt and nickel present in the sample should not exceed 1 mg/l.

Mn 2

The minimum detectable concentration is 5 µg Mn/l with a spectrophotometer, using a 10-cm cell. Up to 1 000 µg Mn/l can be determined directly (1-cm cell). The pH should be between 9 and 10, the optimum range for colour development.

Manganese is made soluble by pre-treatment with potassium peroxodisulphate and treated with formaldoxime. After adjustment of pH with the ammonia solution, the red complex is measured photometrically at 450 nm.

22.3.2 Reagents

Sulphuric acid, about 4.5 M. Add 250 ml concentrated sulphuric acid, H_2SO (sp.gr. 1.84) to 500 ml distilled water. Cool to room temperature and dilute to 1 000 ml.

Manganese standard solution, 100 mg/l. Heat 0.5 g manganese(II) sulphate, $MnSO_4 \cdot H_2O$ for 1 h at 180° C. Dissolve 0.2749 g in distilled water containing 1 ml of concentrated H_2SO_4. Add 2 ml of concentrated formalin to inhibit mould growth, and dilute to 1 000 ml with distilled water. Manganese standard solution, ready for use, can also be purchased.

Iron solution. Dissolve 0.70 g ferrous ammonium sulphate, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, (Mohr's salt) in distilled water. Add 1 ml concentrated H_2SO_4 and dilute to 1 l with distilled water. Store the solution in a polyethene bottle.

Hydroxylammonium chloride solution, 25 percent. Dissolve 250 g hydroxylammonium chloride (H_4ClNO) in distilled water and dilute to 1 l. Store in a polyethene bottle.

EDTA-solution, 0.25 M. Dissolve 93 g disodium-ethylenediammine tetra acetic acid ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$) in distilled water and dilute to 1 l. Store in a polyethene bottle.

Ammonia solution. Dilute 750 ml concentrated (25 percent) ammonia solution (sp.gr. 0.91) with distilled water to 1 l.

Formaldoxime solution. Dissolve 50 g hydroxylammonium chloride (H_4ClNO) in distilled water. Add 25 ml concentrated (40 percent) formalin, HCHO, and dilute to 500 ml. Store the solution in a polyethene bottle. Shelf life 1 month.

22.3.3 Equipment

- Spectrophotometer or filter colorimeter for work at 450 nm.

22.3.4 Sampling and sample pre-treatment

Collect the sample in polyethylene bottles with caps of the same material or in bottles of resistent glass. Preserve the samples by adding 1 ml of the sulphuric acid (4.5 M) per 100-ml sample.

Mix the sample thoroughly and transfer 50-ml to a 200-ml Erlenmeyer flask. Add 0.50 ± 0.01 g of potassium peroxodisulphate, $K_2S_2O_8$, and heat to boiling. Continue boiling until the sample volume is reduced to about 5 ml. Cool to room temperature and transfer the sample quantitatively to a 25- or 50-ml volumetric flask. Treat the blank sample of 50 ml distilled water in the same way.

Alternatively, transfer 50 ml of the sample to a bottle of resistent glass (capacity 100 ml) with a screw cap. Add 0.50 ± 0.01 g potassium peroxodisulphate, $K_2S_2O_8$, screw the cap on and boil in an autoclave or pressure boiler at a pressure of 2 atm (temperature about 120° C) for 30 min. Cool to room temperature. Treat the blank sample of 50 ml distilled water in the same way.

22.3.5 Procedure

A. Fresh water

(1) Treat the sample and a blank (50 ml distilled water) in the same way.

To the sample in the volumetric flask add 2 ml of the formaldoxime solution, mix, and then add 1 ml of the iron solution and 5 ml of the ammonia solution. Mix and wait for three min.

(2) Add 2 ml of the EDTA-solution and 2 ml of the hydroxylammonium chloride solution and dilute to the mark with distilled water. Check that the pH is between 9 and 10.

(3) Measure the absorbance with distilled water in the reference cell, after 30 min at 450 nm. The coloured complex is stable for at least 2 h.

B. Saline water

The analysis of saline water is carried out as described above, with two modifications:

- The blank sample consists of 50 ml synthetic sea water and 0.5 ml of the 4.5 M sulphuric acid. This sample is then treated as the saline water samples.

- Instead of the EDTA-solution, 2 g of the tetrasodium salt of EDTA ($C_{10}H_{12}N_2Na_4O_8 \cdot 2H_2O$) are added to each sample.

22.3.6 Calibration

Prepare a working standard solution by pipetting 5 ml of the manganese standard solution into a 500-ml volumetric flask. Add 5 ml of the 4.5 M sulphuric acid and dilute to the mark with distilled water. Mix well.

Transfer 0 (reagent blank), 5, 10, 20 and 40 ml of the working standard solution to 100-ml volumetric flasks in order to prepare a series containing 0, 50, 100, 200 and 400 µg manganese/l respectively. Add 1 ml of the sulphuric acid and dilute to the final volume with distilled water or synthetic sea water.

Complete the determination as outlined above. Plot absorbance against µg Mn/l.

22.3.7 Calculation

Refer to the standard curve for the manganese concentration and express the results in µg Mn per litre.

22.4 Determination of copper with oxalic acid-bis (cyclohexylidenhydrazide)

22.4.1 Introduction

Most copper minerals are relatively insoluble, and only very low concentrations can be expected from natural sources. The presence of copper in more than trace amounts can usually be attributed to the corrosive action of water on copper pipes, to industrial wastes, or to the use of copper salts for the control of algae and other aquatic growths.

This method is intended for the determination of traces of copper in relatively unpolluted water. It is applicable to drinking water and sea water and is essentially free from interferences. Copper bound to cyanide or organic matter will not be determined. However, chromium(III) will also be determined.

Cu 2

The minimum detectable concentration is 0.02 mg Cu/l with a spectrophotometer, using a 10-cm cell. Up to 12 mg Cu/l can be determined directly (1-cm cell). A pH between 8 and 9 allows full colour development.

Copper ions form a blue-coloured complex with the reagent oxalic acid-bis (cyklohexylidenhydrazide). The coloured compound is water-soluble and is formed quantitatively. A suitable pH is obtained by use of a borate buffer. The complex is measured photometrically at 595 nm.

22.4.2 Reagents

Use redistilled or deionized distilled water.

Sulphuric acid, about 4.5 M. Add 250 ml concentrated sulphuric acid, H_2SO_4 (sp.gr. 1.84) to 500 ml water. Cool to room temperature and dilute to 1 000 ml.

Sodium hydroxide solution, 1 M. Dissolve 20 g sodium hydroxide, NaOH, in water and dilute to 500 ml. Store in polyethene bottle.

Ethanol solution. Add 100 ml ethanol, C_2H_5OH, to 100 ml water.

Copper standard solution, 100 mg/l. Weigh 100 mg copper metal foil or wire (Analar, B.D.H. or equivalent); place it in a 250-ml beaker under a hood; add 3 ml water and 3 ml concentrated nitric acid, HNO_3, and cover the beaker with a watch glass. After all the metal has dissolved, add 1 ml concentrated sulphurid acid, H_2SO_4, and heat on a hot plate. Stop heating just short of complete dryness. Cool and dissolve in water. Transfer quantitatively to a 1-l volumetric flask and dilute to the mark with water. Alternatively, dissolve 0.393 g dry copper sulphate, $CuSO_4 \cdot 5H_2O$, in distilled water, add 10 ml concentrated hydrochloric acid (sp.gr. 1.18) and dilute to 1 000 ml.

Copper standard solutions ready for use can also be purchased.

Buffer solution I. Dissolve 5 g anhydrous boric acid, H_3BO_3, in approximately 150 ml water. Add 20 ml of the sodium hydroxide solution. Check the pH and add dropwise more of the sodium hydroxide solution until the pH is 8.5. Dilute to 200 ml.

Buffer solution II. Dissolve 75 g citric acid, $C_6H_8O_7 \cdot H_2O$, in approximately 100 ml water. Add 75 ml concentrated ammonia solution, NH_3 (sp.gr. 0.91) and cool. Check the pH and add dropwise more of the ammonia solution until the pH is 8.5. Dilute to 250 ml.

Dye solution. Dissolve 0.5 g oxalic acid-bis (cyklohexylidenhydrazide), $C_{14}H_{22}N_4O_2$, in 100 ml of the ethanol solution. If necessary, heat the solution carefully in a water bath. The solution is stable for 2 to 3 weeks.

22.4.3 Equipment

- Spectrophotometer or filter colorimeter for use at 595 nm.

22.4.4 Sampling

Collect the samples in polyethylene bottles with caps of the same material or in bottles of resistent glass. Preserve the samples by adding 1 ml of the sulphuric acid (4.5 M) per 100-ml sample.

22.4.5 Procedure

(1) Transfer 25 ml of the sample or an aliquot to a 50-ml volumetric flask. Add 5 ml of buffer solution I and 10 ml of buffer solution II. Mix.

A reagent blank of 25 ml distilled water is treated in the same way.

(2) Add 5 ml of the dye solution and dilute to the mark with water.

(3) Measure the absorbance, with water in the reference cell, after 45 $\pm$ 10 min at 595 nm.

22.4.6 Calibration

Prepare a working standard solution by pipetting 5 ml of the copper standard solution into a 500-ml volumetric flask. Add 5 ml of the sulphuric acid and dilute to the mark with water. Mix well.

Transfer aliquots 0 (reagent blank) 5, 10, and 50 ml of the working standard solution to 100-ml volumetric flasks in order to prepare a series containing 0, 0.05, 0.10 and 0.50 mg copper/l respectively. Dilute to the mark with water. Use the undiluted working standard solution for preparing a sample containing 1 mg/l.

Complete the determination as outlined above. Plot absorbance against mg Cu/l.

22.4.7 Calculation

Refer to the standard curve for the copper concentration and express the results in mg Cu per litre.

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This chapter has been compiled by B. Röndell.

23. METHODS FOR ANALYSIS OF DDT AND PCB IN ENVIRONMENTAL SAMPLES USING CHROMATOGRAPHIC METHODS

23.1 Extraction, clean-up and separation

23.1.1 Introduction

DDT, its metabolites and PCB, being fat-soluble compounds, are found in the lipophilic part of the sample. To extract the fat, mixtures of organic solvents are used, together with homogenization. The efficiency of the extraction is often controlled by adding a specific amount of DDT or PCB to the sample, carrying the sample through the procedure, and calculating the amount of recovered substance. A blank is also run to check the solvents used. For the advantages and disadvantages of different methods, see Jensen et al., 1973. The fat weight is usually estimated in order to correlate the levels found to the fat weight. This is important when comparing individuals within a species, as well as when studying different species in the food web. A simple method of determining the fat content is to evaporate the organic solvents used for the extraction and weigh the residue.

A clean-up procedure is necesssary in order to have the extracted sample sufficiently free of interfering substances such as fat, as the injection of impure samples will contaminate the gas chromatograph, which is normally used to perform the analysis.

The substances to be analysed which interfere with each other in the gas chromatograph must be separated into different fractions (see Chapter 11).

23.1.2 Fish tissue

Place two 150 ml dropping funnels one above the other, the upper containing a glass filter (Fig. 1). Introduce 10 g of the sample into the upper funnel, add 25 ml acetone and 10 ml hexane. Homogenize the mixture with the insertable homogenizer. Pour 50 ml of a 0.9 percent sodium chloride solution into the lower funnel. Allow the liquid in the upper funnel to run into the lower one. Press down if necessary by means of nitrogen pressure. Close the upper tap. Add 25 ml of a hexane/ether mixture (90:10). Stir the mixture and allow to stand for five minutes, let the liquid run into the lower funnel. Rinse the sample with a new 25 ml portion of hexane/ether.

Stopper the lower funnel and shake the organic solvents/sodium chloride mixture. Remove the stopper and allow the phases to separate. Weigh an Erlenmeyer flask and enter this weight in Table I. Let the lower aqueous phase run into a beaker and decant the upper organic phase into the E-flask. Return the aqueous phase to the lower funnel and shake it with 10 ml of hexane. After separation, add the hexane phase to the E-flask. Pour 10 ml of 99.5 percent ethanol into the E-flask. Place the E-flask in a cold water bath. This is important in order to avoid sudden boiling of the organic phase with consequent loss of the fat solution. Heat the water bath to 90°. When the solvent has evaporated, allow the E-flask to cool and then weigh the flask to obtain the fat weight. Dissolve the fat in a suitable volume of hexane (normally about 50 mg/ml) which contains the internal standards (para 23.2.2). Use the ultrasonic bath if necessary to ensure complete dissolution. Take 2 ml of the fat solution and 4 ml of a concentrated sulphuric acid solution in a glass test tube with a screw cap fitted with a teflon disc. Turn the test tube up and down about 20 times (avoid vigorous shaking) and then centrifuge. Remove the upper hexane layer which is now ready for the gas chromatographic injection.

Alternatively, add to 2 ml of the fat solution in a test tube, 2 ml of 99.5 percent ethanol, 4 pellets of potassium hydroxide and 2 drops of distilled water. Screw on the cap and shake until the pellets have dissolved. Check to ensure that only one phase is obtained. Place the test tube in a water bath held at 50°C for 1/2 h. Then add 6 ml of distilled water, shake and centrifuge. Remove the upper hexane phase, now ready for the gas chromatographic injection.

NOTE: It is important to control solvent interference by running blanks parallel to sample analysis

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